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GROWTH

A JOURNAL FOR STUDIES OF DEVELOPMENT AND INCREASE

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GROWTH

A journal for the coordination of studies of increase and development as general properties of nature. Published by contributors and subscribers on a cooperative, non-profit, non-salary basis. The field of investigation is primary and work will be published in which the approach is through mathematics, physics, chemistry, genetics, embryology, philosophy and anthropology, just as will studies of development in its several expressions, and of mass increase and the part played therein by nutrition, vitamines and hormones.

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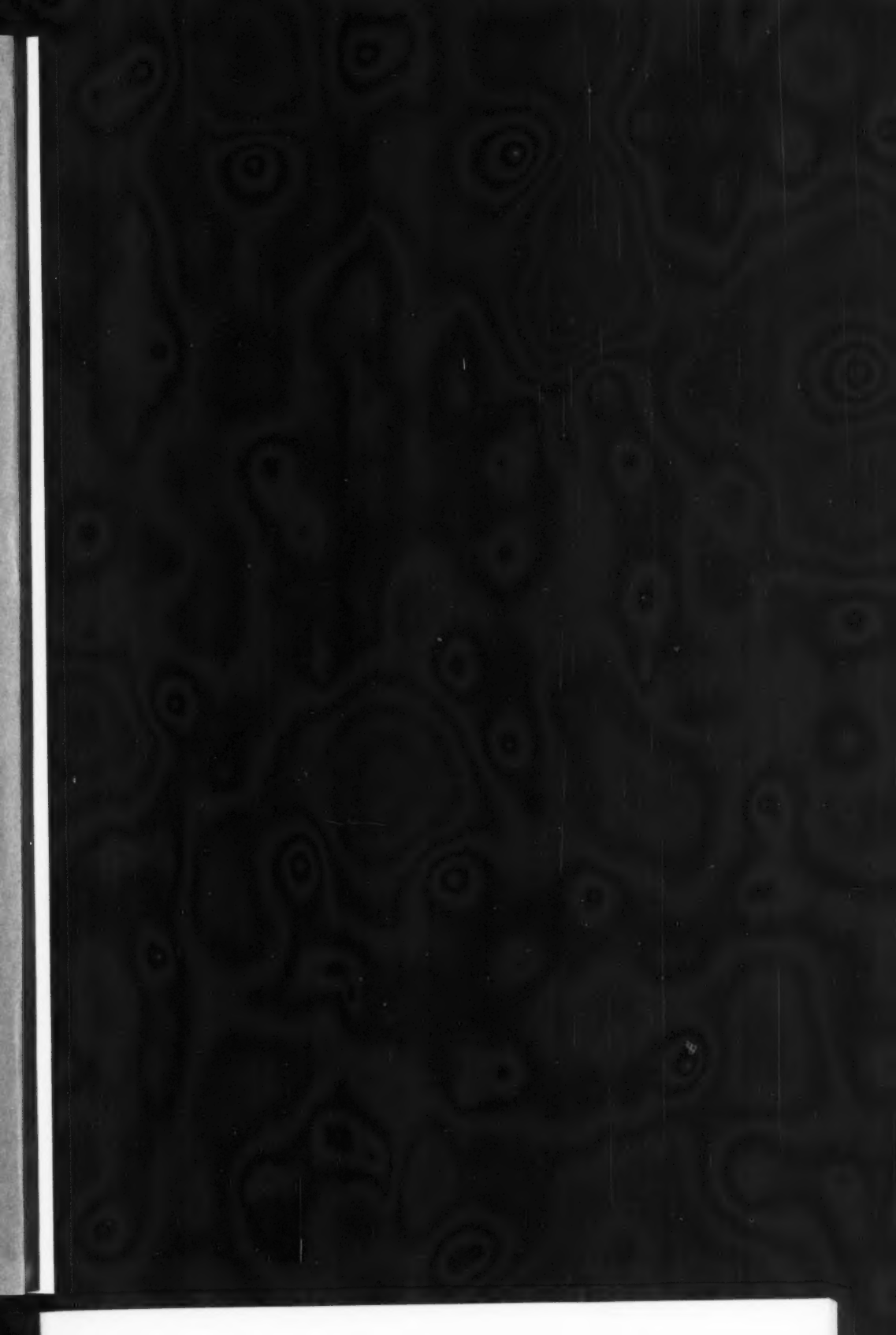

THE EFFECT OF ZINC ON THE GROWTH PROCESS

By
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(*Received February 10, 1939*)

Zea mais (variety Golden Bantam sweet corn) grows very poorly in soil taken at Delhi, California, where little leaf of fruit trees has been an acute problem. Former work in this laboratory has shown that corn growing in such soil is affected adversely by dwarfing, by white bud, and generally, though not always, by an increased production of anthocyan. The symptoms mentioned are shown by plants which are grown in the greenhouse in soil which has been taken up and transported from its original location. Numerous experiments in this laboratory have shown that the condition already mentioned can be corrected by the application of small amounts of zinc sulfate to the soil at the time the crop is planted and at proper intervals thereafter.

The experiments here to be related were conducted to find out how the lack of a suitable amount of zinc affects the growth process of the plant. For this purpose metal tanks containing about 50 kilos of soil were used. There were ten tanks of soil, with ten plants in each tank. Five of the tanks received no zinc; the remaining five had small amounts of zinc sulfate added. The first crop of plants grew from June 28 to October 4, 1937; the second crop from April 21 to July 12, 1938; the third crop from July 14 to October 15, 1938.

At the beginning of the experiment, each tank received an application of KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$, and a trace of boron. After the plants had grown for approximately a month, additional $Ca(NO_3)_2$ was applied, and repeated subsequently. In experiment number one, the applications of zinc sulfate were .2 gram per tank on June 26, July 27, and July 31. In the second experiment, the amounts were 1 gram each, applied April 20, April 26, and June 4. In the third experiment, the amounts were also 1 gram, which were applied July 13, August 11, and September 7. The soil was irrigated with distilled water at frequent intervals, to maintain a good moisture condition.

As soon as the young plants were started, they were labeled and measurements began when they were approximately 10 to 15 cm. in height. Measurements were made to the tip of the tallest leaf, until

the tassel surpassed the tallest leaf, when the tip of the tassel was used. The height of plants in the first crop was measured each five days; in the second and third crops, each seven days. In each experiment there was some apparent relation to the amount of light which the various tanks received. For example, in the second experiment in the series

FIG. 1. Corn plants from one of the experiments in Delhi soil. The plant on the left grew in soil to which zinc sulfate was supplied in addition to fertilizer salts; the extremely dwarfed plant on the right grew in soil to which no zinc was supplied although the same application of fertilizer salts was given.

lacking zinc, the best plants were in two tanks near the center of the house furthest from the side wall, but the tank ranking third was one at the end next to the side wall, where it undoubtedly received more light. The poorest tanks were in the intermediate position. The series of tanks which received zinc showed rather different relations. The best tanks were those next to the side wall, where the amount of light

received was greatest; the middle tank in the row was intermediate; and the inner were the poorest. For the time being, the differences are referred to the different light intensities. The experiments were conducted during the summer when the windows and doors were open most of the time, and it is not likely that there was any great amount of difference in the temperatures of the various tanks.

TABLE I

GENERAL RESULTS SHOWN BY FIRST, SECOND, AND THIRD CROPS

AVERAGE FINAL HEIGHT (CENTIMETERS)

	Minus zinc	Plus zinc
First Crop	140.4±2.2	155.9±1.9
Second Crop	128.4±1.7	154.7±2.2
Third Crop	97.7±1.5	124.8±2.0

AVERAGE DRY WEIGHT OF PLANTS (GRAMS)

	Minus zinc	Plus zinc
First Crop	41.6±1.8	50.9±1.8
Second Crop	33.9±1.0	42.7±1.4
Third Crop	19.7±0.7	25.2±1.1

In the absence of zinc there is pronounced dwarfing of the corn plant, as shown in the photograph of plants taken from the first crop (Fig. 1). The plant on the left grew in Delhi soil to which zinc sulfate had been added, and the extremely dwarfed plant on the right grew in another lot of the same soil to which no zinc was added. The number of leaves on the two plants is the same, and the size of homologous leaves is not greatly different. The greater height is principally due to the greater length of the internodes. Many leaves of the plants in the zinc-deficient soil showed banded chlorosis identical with the condition described by Hoagland, Chandler, and Hibbard (1936). The shortened internodes of fruit trees are one of the characteristic features of zinc deficiency, (Reed, 1937). One of the problems of this investigation was to find the effect of the zinc salt on the process of reproduction.

The general effect of the application of zinc to this soil is shown by the final height and dry weight of the plants in the three crops. It is apparent from table 1 that the differences attributable to zinc increased in successive crops. It will be noted that repeated cropping had the

TABLE II

AVERAGE DRY WEIGHTS OF PLANT MEMBERS

	Stalks	Leaves	Husks	Cobs	No. of plants having cobs with Kernels
First crop					
minus zinc	20.5±0.9	8.5±0.3	10.6±0.6	2.0±0.2	14
plus zinc	25.0±0.9	8.7±0.2	13.4±0.8	3.3±0.3	12
Second crop					
minus zinc	16.1±0.5	7.8±0.2	6.3±0.3	4.1±0.5	22
plus zinc	20.4±0.7	8.2±0.2	5.1±0.2	11.0±0.7	36
Third crop					
minus zinc	9.7±0.4	4.5±0.1	3.6±0.2	1.8±0.3	14
plus zinc	10.3±0.3	5.3±0.2	3.6±0.3	5.9±0.6	33

effect of reducing the yield of the plants in each case. The dry weight of the plant members also showed interesting differences in the two series. The figures in table 2 show the decrease in dry weight of stalk, leaves, and husk in the succeeding crops. The difference in weight of

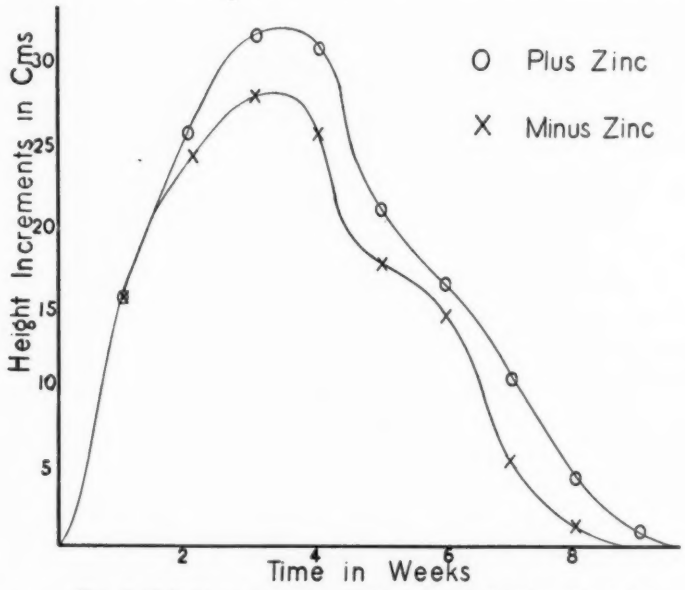

FIG. 2. Velocity curves of growth of plus and minus-zinc series from crop 2, showing height increments at weekly intervals.

stalk attributable to zinc was well marked in two of the three crops, but not in the weight of leaves. In the same way the difference in weight of husks was well marked in two of the three crops. The weight of cobs was in all three crops much greater where zinc had been applied to the soil and in the second and third crops the number of cobs bearing kernels was much greater where zinc had been applied.

The rate of growth of the two series of plants in the second crop is shown in figure 2. Measurements of this crop started on May 3 and were continued for eight weeks thereafter. It will be seen from the figure that there was little difference in the weekly elongation of plants in the two series until the third week. After that there was always less growth in the minus-zinc plants. It will also be noted that the difference between the two series of plants was greatest when they were making their greatest growth, and that the growth period of both series was practically the same. One might account for this type of curve by assuming that the seeds contained enough zinc to last the plant for a time in the minus-zinc soil, and that the lag began to show when the original supply of zinc had become inadequate for growth. In the fourth and sixth weeks, additional calcium nitrate was given to both sets of plants.

TABLE III

AVERAGE HEIGHT OF CORN PLANTS

Time Weeks	Minus Zinc		Plus Zinc	
	Observed	Calculated K = 0.050	Observed	Calculated K = 0.055
	Cms.	Cms.	Cms.	Cms.
1	15.0	13.2	15.1	12.4
2	39.0	34.7	39.9	35.8
3	66.6	62.7	71.5	69.8
4	92.1	89.5	102.4	104.6
5	109.4	108.4	123.3	130.3
6	123.6	119.5	139.3	144.3
7	128.1	125.1	149.9	151.0
8	128.6	127.8	153.8	153.9
9	128.5	128.1	154.7	154.7

The curves of growth (fig. 3) of the two sets of plants were calculated from $\log \frac{x-\alpha}{A-x} = \log \frac{\alpha}{A} + K\ (t-t_1)$ where A = final height, t_1 = time when the growth was half attained, α = the value of x at zero time, and K = constant (Reed, 1932). The plus-zinc plants made slightly less growth than the theoretical curve would indicate in the fifth, sixth, and seventh weeks (table 3). Otherwise, the agreement

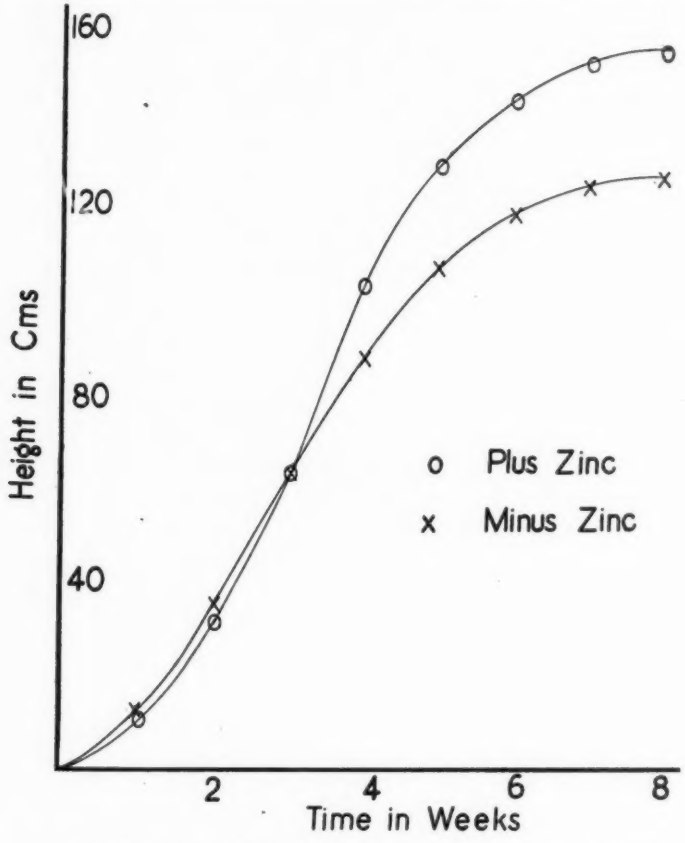

FIG. 3. Curves of growth of plus- and minus-zinc series from crop 2, calculated from the following equations:

Plus zinc $\log \frac{x+10.1}{156-x} = \log \frac{10.1}{156} + 0.055\ (t-23)$

Minus zinc $\log \frac{x+13.0}{130-x} = \log \frac{13.0}{130} + 0.050\ (t-21)$

between the observed and the calculated values was satisfactory. It will be observed that the value of K (the intensity of growth) was somewhat larger in the case of the plus-zinc plants, although the difference between the two is not very great.

The calculated values of the integral curves do not separate widely until after the third week. This may mean that the seeds when planted had a supply of zinc sufficient to carry the plants for a time.

SUMMARY

1. Maize plants grown in Delhi soil without the addition of zinc showed deficiency symptoms comparable to those obtained in culture solutions of purified salts.

2. Height and dry weight of plants showed consistent differences in favor of small amounts of zinc in all three crops.

3. A deficiency of zinc curtailed the production of cobs and kernels more than of stalks, leaves, and husks.

4. The grand period of growth of the two sets of plants was practically the same.

5. The equations for the growth curves of the two lots of plants resemble each other, although the values of the constants representing final height and half-time are greater in case of the plus-zinc series.

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NOTES ON AN AFRICAN ELEPHANT (ELEPHAS LOXODONTA AFRICANA)

By
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(*Received February 11, 1939*)

During the winter of 1935-36 members of the Cleveland Clinic-Cleveland Museum of Natural History Expedition to Tanganyika Territory and Uganda secured a large bull elephant after a ten day hunt for this particular specimen. Notice was first called to this beast by Captain J. R. H. Hewlett, our professional guide, who picked up the trail of this animal from time to time during our hunt for other African specimens. From the nature of the animal's spoor Captain Hewlett deducted that he was a large bull, perhaps just beyond his prime and pushed from the herd by younger bulls. From measurements of the tread which had a diameter of 23 inches for the forefoot he inferred that the animal was of exceptional size. When we were ready to take up the dissection of the elephant we decided to secure this particular animal if possible and our tracking and final success in acquiring "Tembo", as we christened him, was one of the most interesting chapters of our African experience. After following his spoor for two days he seemed to become aware that he was being hunted and the ensuing eight days represented a battle of wits and strategy between "Tembo" and the hunters.

The animal was finally brought down by Dr. George Crile, in a dried stream bed where he was skinned, examined and weighed as he had fallen. In view of the dearth of measurements on the African elephant it is hoped that the information we secured may be of some value.

The beast was a full-grown male. Estimates of his age varied; Captain Hewlett estimated it to be between 70 and 80 years while the native trackers considered him older. The old hunter's rule of a pound per tusk per year has little meaning in view of the great difference in the amount of ivory carried by the various African elephants in different parts of the continent. The fact that our bull had been supplanted in the herd by younger animals may have meant that he was beyond his prime although none of his organs showed any changes due

to old age. His tusks and dentition were perfect and he carried no scars on his body.

The animal fell on his right side and the body was entered from this side. The mechanics of the dissection were formidable in view of his enormous bulk. His length from tip of trunk to tip of tail was 24 feet and 4 inches; the tail alone was 4 feet, 9 inches in length. His circumference at the back of the shoulders was 15 feet, 8 inches. His height at the shoulder was 10 feet, 10 inches. The circumference of the front foot at the wrist was 60 inches and the back foot measured 57 inches at the ankle. The length of the tusks from the point where they emerged was 43 inches; at their base they measured 17 inches in circumference. The distance from the eye to the tip of the proboscis was 8 feet, 6 inches.

The weights of the body parts were taken with a Cenoco balance and a Chatillon autopsy scale for the small glands and organs and with a Chatillon spring scale, 600 pound capacity, for the larger portions. The pituitary was weighed with a fine assay balance. While a factor of error was introduced for the weights of the larger parts, in view of the large number of individual weighings, great care was exercised and the same individuals did the weighing throughout the task. The following are weights recorded:

WEIGHTS OF BODY PARTS OF AFRICAN ELEPHANT*
(WEIGHT IN POUNDS)

Part	Weight	Part	Weight
Muscle	7712	Heart	57.5
Skeleton	1238	Kidneys	40.0
Skin	2265	Testes	19.4
Intestines and Contents	2034	Brain	12.5
Liver	235	Thyroid	1.89
Lungs	306	Adrenals and Adnex.	4.84
Genitalia	69	Eyes	.55

* Total weight allowing 5% for seepage and blood and deducting 50 pounds for sack and rope employed in weighing. This is an arbitrary deduction but the estimate is generous since many of the pieces were hung directly on the hook of the scale without using rope or sack.— *14,640.53 pounds*

DISTRIBUTION OF WEIGHT

Muscle: The total muscular weight of the animal was 7712 pounds, of which 524 pounds belonged to the head, proboscis and ears; approximately 393 pounds represented the musculature attached to the ribs and the vertebral column after most of the epiaxial musculature had been removed, thus leaving 6795 pounds of musculature to be

distributed among the four extremities, the back, and the neck. I would judge that this mass was distributed about as follows: 1250 pounds for each of the limbs, including feet and shoulders or hip attachments and approximately 1795 pounds distributed along the vertebral column as epiaxial, psoas and iliac and scalene musculature. If there is an error in this latter distribution I believe it would lie in the direction of under-estimating the weight of the limbs.

Skeleton: Our original weights included the skeletal mass hidden in the musculature, since time did not permit separation of muscle and skeleton. The body and the limbs were cut up in convenient portions for weighing, i.e., in pieces weighing under 600 pounds, since that was the capacity of our scale. The skeleton was allowed to weather for some ten days, after which it was weighed. Some cartilages and foot bones were lost during the removal of muscle by our native "boys".

The weights are as follows:

Cervical and lumbar vertebrae	153	pounds	
Ribs and thoracic vertebrae	111	"	
Scapulae	74	"	
Femurs	122	"	(Distal end of one chewed)
Tibiae and Fibulae, all	54	"	
Radii and ulnae, all	48	"	(Distal end of radii missing)
Humeri	110	"	
Pelvis	61	"	(Iliac margin chewed)
Tusks, right 41, left 44	85	"	
Skull	220	"	
Lower jaw	60	"	
*Foot bone from wrist and ankle down estimated at:	120	"	(Unfortunately these were lost, the feet were placed in the hands of our native skinners and they saved the skin but lost the bones.)
Estimated weight of lost bits	20	"	
	1238	pounds	TOTAL

This does not include tail vertebrae.

Skin: The skin of the body exclusive of the skin of the head and the skin of the limbs from the knees and the wrists down weighed 2013 pounds. This item included, however, the weight of the tail with caudal vertebrae. I estimate the actual skin weight of the tail to be 5½ pounds, and 2½ pounds on this basis would be assigned to caudal vertebrae, muscle and connective tissue. The estimated skin weight of the skull, if it followed the same proportions as found on the body,

would amount to 105 pounds. Actually I think that this is slightly low, since the enormous surface area of the ears and proboscis should be taken into account. This is as close as we can estimate it, however. The skin of the feet in the fresh condition weighed 150 pounds. In the fresh condition the skin of these parts weighed with the nails, 39 pounds each for the front limbs and 36 pounds for each hind limb. The total weight of the skin then should be 2,265 pounds.

Intestine: The intestinal mass, including esophagus and stomach, as well as contents, weighed 2034 pounds. Again, the amount to be ascribed to actual weight of the system exclusive of contents must be estimated. Our estimate is 1100 pounds for intestinal and stomach contents and 934 pounds for weight of the system itself. The stomach and intestine contained large amounts of food. The colon was partly filled with fecal material in the shape of large boluses of undigested matter weighing from ten to fifteen pounds per bolus. Superficial examination of the tract failed to disclose intestinal parasites.

Liver: The portal vein was 4½ inches in diameter. The liver possessed well-marked right and left lobes with smaller caudal lobule. The entire weight of this gland was 235 pounds.

Heart: The total weight of the heart was 57.5 pounds. The left ventricle terminated in a marked tip rather than in blunt ending. The heart was caught in complete atrial systole. Four pulmonary arteries opened into the left atrium. Both interatrial and interventricular septa were thick and muscular. The interatrial septum was 5/16 of an inch in thickness. The coronary artery was ¾ inch in diameter, permitting easy ingress of my thumb. The aorta at emergence from the heart was 4¼ inches in diameter.

Kidneys: These were of the lobulate type, weighing a total of 40 pounds, with the weight equally distributed.

Lungs and larynx: The larynx was separated from the trachea at the level of the first cartilage below the cricoid. The weight of the larynx and hyoids (which were attached to the skull by articulation) was 16 pounds and the lungs and trachea together weighed 306 pounds, making a total of 322 pounds for the lungs and larynx.

Penis and testes: The pendulous portion of the penis weighed 44 pounds and the internal portion, i.e., the bases of the cavernous portions of the gland, weighed 25 pounds more, making a total of 69 pounds. The right and left testes together weighed 19.4 pounds. To this weight

the right contributed 9.15 pounds and the left 10.25 pounds.

The brain: The brain and pituitary together weighed 5712.36 grams. The pituitary with dura contributed 12.36 grams and the brain proper the balance. The total weight was 12.59 pounds.

Thyroid: The two halves of the thyroid gland were closely joined by connective tissue. The combined weights of these two portions was 860 grams. The total was 1.90 pounds.

Adrenals: These glands were removed with their adnexa from the dorsal wall of the body cavity. The total weight with adnexa was 2200 grams. The weight of the glands alone and freed from these portions was 940 grams or 2.07 pounds.

In addition to this there was an additional mass of 3570 grams or 7.87 pounds of fat about the adrenals.

Eyes: The weight of the eyes with muscles closely trimmed to the eye balls and after removal of all ophthalmic fat was 97.6 grams. Untrimmed they weighed 134.7 grams. (.275 pounds).

Salivary glands: These glands, including submaxillary and sublingual, weighed a total of 8100 grams, 17.86 pounds.

From the total weight secured which is 13,958.65 pounds, a deduction of 50 pounds should be made for rope and the bag employed in weighing. Considering this amount as 95 per cent of the total weight, the additional 5 per cent would bring up the total weight to 14,640.53 pounds.

In connection with the weight of this animal, the following paragraph from a letter by Francis G. Benedict, Director of the Nutrition Laboratory of the Carnegie Institution, may be of interest:

"Your total corrected weight of 14640 pounds certainly is the record for anything that can be said to be scientifically accurate. The nearest thing to it is that reported in the elephant book for Bolivar of 12,000 pounds."

SUMMARY

An elephant which subsequently was found to weigh 14,640 lbs. was shot in Tanganyika Territory, South East Africa on December 27, 1935.

In the course of the dissection weights of skin, skeleton, muscle, viscera, glands, and brain were secured. These are recorded in the hope that because of the great size of the animal and its parts, they may be of value for comparative purposes.

THE INFLUENCE OF CERTAIN INORGANIC SALTS ON TUMOR TRANSPLANTS

By
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(*Received February 13, 1939*)

Several investigators (1) (2) (3) have studied the influence of diets on the growth of the various types of tumor transplants. Moreschi (4) observed that grafts of the mouse sarcoma grew less frequently and more slowly in animals losing weight on a low diet. On the other hand Rous (5) found that the Flexner-Jobling carcinoma was not influenced by under feeding.

The status of organic salts relative to tumor growth is dependent upon clinical observations and some experimental studies. In France, cancer of the lung is recognized as an occupational disease in the cobalt mines (6). Injections of lead compounds (7) have been reported favorable for inhibiting tumor growth but unfavorable because of the toxicity to the host. McCarrison (8) found that the daily administration of .009 mgs. of magnesium per rat accelerated the tumor growth, however, .056 mgs. daily inhibited the growth, Collier and Cohn (9) claimed that the subcutaneous administration of sodium chloride increased tumor growth while an equivalent quantity of calcium had no effect.

TABLE I

Diet Number		Casein	Starch	Butter	Yeast	Cod Liver Oil	Salt Mixture	$Ca(OH)_2$	$CoSO_4$
1	Salt Deficient	18	50.00	15	15	2	0	0	0
2	Standard	18	47.00	15	15	2	3	0	0
3	$CoSO_4$	18	49.95	15	15	2	0	0	.05
4	$Ca(OH)_2$	18	48.00	15	15	2	0	2	0

For our study of the influence of certain inorganic salts on the growth of tumor transplants, a basic diet (Table 1) was selected which was easily modified in its organic constituents. Modifications were made with a minimum of disturbance to the nutritional value relative

to the protein, fat, carbohydrate, and vitamin content. The basic diet (diet 1) consisted of casein, starch, butter, yeast and cod liver oil. The standard diet (diet 2) was a modification of the basic diet in which 3 percent of the starch was replaced by an inorganic salt mixture (Harris). The inorganic salts selected for study were cobalt sulphate (diet 3), and calcium hydroxide (diet 4). The cobalt sulphate diet was prepared by substituting .05 percent cobalt sulphate for an equivalent quantity of starch in the basic diet. The calcium hydroxide diet was similiarly prepared from the basic diet by substituting 2.0 percent calcium hydroxide for an equivalent quantity of starch.

The rats used in this study were taken from our own colony which is maintained on a diet of Fox Chow *ad libitum*, with the addition of bread, meat, and cabbage once a week. The animals were weaned at the age of 21 days and continued on the above diet until selected for the experiments. Only males were used in this study. Littermates between the ages of 26 and 31 days were divided into five groups. The first two groups (24 animals) received the standard diet (diet 1). The third group (29 animals) received the salt deficient diet (diet 2), the fourth group (11 animals) the $Ca(OH)_2$ diet (diet 3), and the fifth group (21 animals) the $CoSO_4$ diet (diet 4). After being on their respective diets for seven days the animals were anaesthetized and two small pieces of viable spindle cell sarcoma (10) were placed beneath the skin. The technic consisted in making a dorsal transverse incision about one centimeter long through the skin at about the level of the third lumbar vertebra. The skin was then separated from the underlying tissue between the lateral limits of the incision and the midventral line with a small hemostat. The tumor tissue was then placed in the pockets thus made and the incision closed with a continuous suture. The $CoSO_4$ used in diet 4 was found to be too toxic to obtain a sufficient longevity to allow for a study of tumor growth. For this reason the diet was given alternately with the basic diet. The alternations were sufficiently frequent to prevent gain in weight and yet permit an average span of life of 21 days. The first group consisted of 8 animals in which the tumor transplants failed to grow, these animals were kept on the standard diet as controls for the other groups.

All animals were weighed at weekly intervals and at the time of death or as shortly thereafter as possible. The average total change in weight per rat was obtained from the weight of the animal at the time

of inoculation and the weight after death when the tumor tissue was removed. The average weight of the standard tumor bearing animals was 41 percent below the average weight of the control, non-tumor bearing, animals receiving the same diet. The group receiving the basic diet was 51 percent below the same standard group, while the Calcium Hydroxide and Cobalt Sulphate groups were 60 and 71 percent respectively below this standard. The failure of the animals on the basic, calcium hydroxide, and cobalt sulphate diets to maintain a weight curve equal to that of the standard tumor bearing animals was obviously due to deficiency or toxicity of the diets.

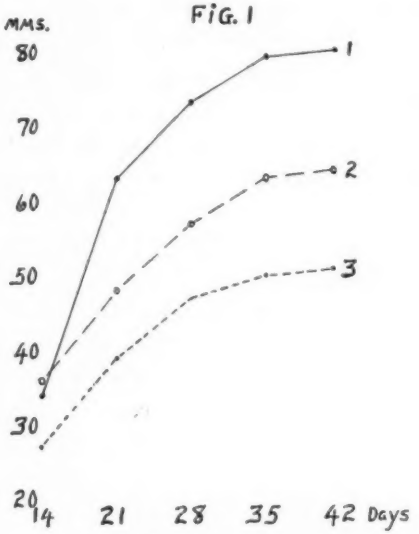

FIG. I Tumor measurements in milimeters.
1. Animals on a standard diet.
2. Animals on the salt deficient diet.
3. Animals on the calcium hydroxide diet.

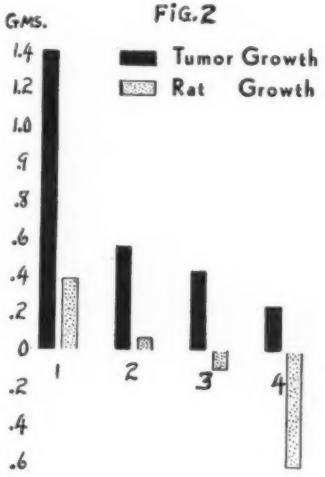

FIG. II A comparison of the average daily change in weight of tumor to that of the host.
1,2, and 3, same as in Fig. I.
4. Animals on cobalt sulphate diet.

Weekly measurements were made along the long axes of the tumor with a sliding caliper. These measurements, of course, included two layers of skin, which, however, were constant from week to week and comparable in all animals. The weekly measurements on the group receiving the cobalt sulphate diet was not significant because of the short survival period. The curves (Fig 1) obtained on the remaining

groups revealed the greatest rate of tumor growth in the group receiving the standard diet. While the slowest rate of growth was found to be in the group receiving the calcium hydroxide diet.

The average daily growth of tumor tissue was obtained by dividing the total weight of the tumors by the average duration of the experiment, likewise, the average daily change in weight of the host was obtained by dividing the total change in weight of the rats by the duration of the experiment. The assumption in this method that the daily growth would be the same for different periods in development is obviously not true (Fig. 1), however, figures thus obtained are comparable. The average daily change in weight of tumor bearing rats (Fig 2) varied from a gain of approximately .4 of a gram in the standard group to a daily loss of .6 grams by the group receiving the cobalt sulphate diet. The average daily growth of the transplant bore a close direct relation to the daily change in weight of the host. The most rapid daily growth being in the group which received the standard diet while the slowest growth occurred in the group receiving the cobalt sulphate.

At the close of the experiment the average weight of the tumor tissue removed from each rat on standard diet was approximately 57 grams, while the average weight of tumor tissue removed from each animal on the cobalt sulphate diet was approximately 5.0 grams. The average weight of tumor tissues from each animal on the salt deficient diet and the calcium hydroxide diet was 23.0 and 18.0 grams respectively. It is obvious from these figures that the more deficient or toxic the diet the less the tumor growth.

SUMMARY

1. The growth of spindle cell sarcoma (McJunkin) transplants retards the growth of the host.

2. The average survival period for tumor rats varying in age from 26 to 31 days receiving a standard diet was found to be approximately forty-one days.

3. The average survival period for tumor bearing rats on a salt deficient diet or on a diet containing 3 percent calcium hydroxide as the only supplementary salts was found to be the same as that for tumor bearing animals receiving the standard diet.

4. Tumor transplants grew larger and more rapidly on animals

receiving the standard diet than on those receiving either the salt deficient, calcium hydroxide or cobalt sulphate diets.

5. A close relation exists between the influence of the diets studied on the growth of tumor transplants and their influence upon the weight of the host.

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GROWTH OF MICROORGANISMS IN SHAKE CULTURES UNDER INCREASED OXYGEN AND CARBON DIOXIDE TENSIONS

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(*Received February 14, 1939*)

The object of this paper is to discuss findings obtained in growing microorganisms in shake cultures under increased gas tensions and to correlate the appearance of the growths with precipitates in gels. This method of growth should take its place with aerobic and anaerobic methods in bacteriological investigations. Changes are of the same order, and are discussed without reference to individual organisms, except in illustration, where certain organisms showed varied changes or examinations were involved.

In previous papers (Williams, 1938, a, b) subsurface growths developing under increased gas tensions were designated bacterial growth "spectrums." The term spectrum is used since many organisms showed characteristic bands or lines resembling those in spectrums of metals. It was found that depth of the upper line of spectral growth increased almost proportionately with increase in oxygen tension (3-160 lbs.) but density, type and width of bands did not change. Depth also increased almost proportionately with increase in carbon dioxide tension but density and width of band varied (see text). Increase in nitrogen (12-160 lbs.) changed neither depth nor width of spectrum. Depth of upper limit of growth increased with decrease in nutrient (8-0.5 gms. nutrient broth Difco/1,000 cc.), while width increased almost logarithmically with depth. Nutrient broth was used since amino acids (asparagin, d-glutamic acid) gave less consistent results. An agar concentration of 7.5 gms./1,000 cc. was used since higher strengths did not give as clear a medium.

APPARATUS AND MATERIAL

Organisms studied are listed numerically in the table and paper. Other abbreviations used are: NB, nutrient broth per 1,000 cc.; O_2, oxygen; CO_2, carbon dioxide.

Contribution No. 147 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

Mediums adjusted to pH 7.2 were put up in 12-15 cc. shakes in bacteriological test tubes, melted, cooled to 40-45°C., planted with 0.2 cc., 24 hour nutrient broth culture, rolled between the hands and plunged in running tap water to solidify. The chilled tubes were placed in the pressure chamber. This gave an initial temperature of 21°C., gradually rising to 37°C.. Except where designated, shake cultures contained 2 gms. NB per 1,000 cc. and were incubated at 37°C. for 48 hrs. under 60 lbs. oxygen pressure.

An even distribution of organisms was shown by plugging glass tubing at one end with a cork and using it like a test tube, replacing the cork with a cotton plug after the medium had been planted and solidified, since typical spectrums equidistant from each end occurred on incubation under increased O_2 pressure. Likewise, rolling and shaking the planted tube violently for $\frac{1}{2}$ minute did not change depth or type of spectrum.

The apparatus consisted of a heavy iron pressure chamber with a pressure gauge, chamber for a liquid pressure type recording temperature capsule and a valve for regulating gas. A flexible metal tube connected the temperature capsule to a clock temperature recorder hung on framework of apparatus. The inner compartment held 150 test tubes. Commercial tanks provided gas. The chamber was not exhausted and always contained atmospheric carbon dioxide, oxygen and nitrogen.

A plate and a table are given. Since depth of upper limit of growth and width for all organisms show like tendencies they were averaged and a "spectrum index" was determined by using former as numerator and latter as denominator. This allowed comparison of effects of various gas pressures and mediums. Depth and width of growths were measured from bottom of meniscus of medium.

RESULTS

Depth of Growth. Depth of upper limit of growth was dependent on oxygen tension, carbon dioxide tension, time of growth, temperature of growth and its initiation, number of organisms planted, percentage of nutrient, factors inhibiting growth, reducing and oxidizing agents.

The almost proportionate increase in depth of upper limit of growth produced by increase in oxygen tension has been considered previously. The greater increase in depth by increase in carbon dioxide tension has

not been considered in detail. The following tensions of CO_2 and O_2 (spectrum index measurements in parentheses) were used: 30 lbs. CO_2 $\left(\frac{3}{?}\right)$; 60 lbs. CO_2 $\left(\frac{8.5}{?}\right)$; 20 lbs. CO_2, 12 lbs. O_2 $\left(\frac{5}{3.3} = 1.52\right)$; 20 lbs. CO_2, 60 lbs. O_2 $\left(\frac{8.5}{5.5} = 1.45\right)$; 30 lbs. CO_2, 30 lbs. O_2 $\left(\frac{7}{4.4} = 1.6\right)$; 30 lbs. CO_2, 60 lbs. O_2 $\left(\frac{10.3}{4.7} = 2.2\right)$; 50 lbs. CO_2, 50 lbs. O_2 $\left(\frac{10.5}{5.9} = 1.79\right)$; 60 lbs. CO_2, 60 lbs. O_2 $\left(\frac{11.8}{3.87} = 3.05\right)$; 100 lbs CO_2, 60 lbs. O_2 $\left(\frac{18.5}{2} = 9\ 25\right)$; 50 lbs. O_2 $\left(\frac{4.4}{5.4} = .85\right)$; 60 lbs. O_2 $\left(\frac{4.64}{6.4} = .725\right)$; 100 lbs. O_2 $\left(\frac{6.5}{5.63} = 1.12\right)$; 150 lbs. O_2 $\left(\frac{9}{6.5} = 1.38\right)$ Increase in carbon dioxide tension inhibited growth especially when increased out of proportion to that of oxygen. Inhibition of growth was great and width of growth indefinite under 30 and 60 lbs. CO_2. Increase in oxygen tension decreased inhibition and increased width of growth. Increase in carbon dioxide seemed to increase depth of growth twice as much as oxygen plus that of inhibition of growth. This raises the question, does CO_2 modify the physical state of the medium out of proportion to but in certain respects similar to O_2 (Williams, to be published).

Certain organisms (6 hr. to 5 day cultures) grown under increased oxygen pressure seem to initiate growth in lines at one or more depths and given time, growth extends above and below these lines. With certain organisms this growth may occur as granules or a decreasing turbidity. Growth extends to a greater extent below than above the spectrum, some organisms developing bands. As time increases the extension decreases.

Depth of growth is dependent on temperature of incubation, temperature of tubes when pressure is applied and temperature of initiation of growth. The upper limit of growth was several millimeters deeper when tubes were inserted in the chamber at 21°C. than when they were inserted at 33°C. While the type of spectrum is not markedly changed the upper and lower delimitations of many growths inserted at 33°C. were less definite (1, 2, 3, 5, 6, 7, 8, 10, 70x) and a mist of growth

TABLE

	A	B	C	D	E	F
1. E. coli communis	3-15	11-26½	4-15½	1½-6	8-28	8-11
2. B. subtilis	2½-5	10-12	12-13	½-2	6-12	10-13
3. Staph. aureus	5-9	11-17	16-17	4½-6	13½-21	14-22
5. Serratia marcescens	2½-6	8-14	2-5½	1-4	5-12	16-16½
6. Ps. fluorescens	2-8	8-17	1½-7	½-3½	6-19	10-15
7. Ps. aeruginosa	2-8½	8-19	2-9½	1-4	6-20	10-15
8. Aerobacter aerogenes	1½-10	8-20	2-10	1-4½	6-23	8-13
9. B. megatherium	4-6	12-14½	15-16	2-5	8-14	12½-15
10. Proteus vulgaris	4-9½	12-14	5½-9	2-5	9-24	13-15
13. B. niger	2½-5	11½-13½	11-12½	2-5	6-11½	12½-15
14. B. mycoides	2½-6	9½-13	3-7	2-5	5-7	17½-20
21. Staph. albus	11-14	20½-22½	17½-18½	6-7	14-17	16-17
45. Proteus X-19	6½-11	20½-24	7-9	6-9	12-25	20-21
52. B. anthracis (attenuated)	1½-3	10½-14	3-5½	1½-4	6-8	15-20
70x. B. enteritidis	4-13	12½-25½	4-12½	2½-7	7-27½	11-17
Index	$\frac{3.63}{5}=$	$\frac{11.5}{7}=$	$\frac{7}{4.13}=$	$\frac{2.3}{3}=$	$\frac{7.87}{9.93}=$	$\frac{11.8}{3.87}=$
	.730	1.64	1.7	.770	.778	3.05

EXPLANATION OF TABLE

Mediums represented in table are: A) 2gms. NB, 7.5 gms. agar/1,000cc., B) 2gms. NB, 7.5 gms. agar/1,000cc., 160 lbs. O_2, C) 2gms. NB, 3mgm. methylene blue, 7.5 gms. agar/1,000cc., D) 2gms. NB, 2.5gms. sodium lactate, 7.5gms. agar/1,000cc., E) 0.5gms. NB, 7.5gms. agar/1,000cc., F) 2gms. NB, 7.5gms. agar/1,000cc., 60 lbs. O_2, 60 lbs. CO_2. A, C, D and E were grown under 60 lbs. O_2. First number in results indicates depth of upper limit of growth from bottom of meniscus and the second the lower limit of

frequently extended for 35-60 mm. into the medium below. When the tubes were inserted cold such a mist was frequently only apparent with 1. When dextrose (1%) was added to the medium in either instance the mist was markedly accentuated and the delimitations masqued to absent.

The greater solubility of oxygen in cold solutions may apply to mediums and account for at least some of this mist and increase in depth. It seems rapid establishment of solid bands of growth (evident in 24 hr. cultures) occuring in optimum oxygen tension range inhibits growth especially above. By cutting off the section of medium above and below such a band of growth, tubing this medium, autoclaving and replanting with the same organism (70x, 10) approximately normal growth occured in medium from below the growth but a faint mist of growth in the medium from above the growth.

As nutrient in medium is decreased (from 8 to .5 gms. NB) the upper limit of growth became deeper. This may have been due in part to decrease in reducing substances. Increased oxygen permeability of medium with wider optimum range for microorganismal growth may occur with decrease in nutrient. The interrelationship of oxygen in medium and reducing substances is not understood. Importance of permeation of oxygen is suggested since planted shakes first grown at atmospheric pressure for 48 hrs. (these growths are usually somewhat scant and occur within 2 mm. of the surface) when subsequently grown under 60 lbs. oxygen for 48 hrs. $\left(\frac{9.3}{5.1} = 1.82\right)$ give a growth spectrum resembling planted shakes grown only under 60 lbs. pressure for 48 hrs. $\left(\frac{3.633}{5} = 0.730\right)$ but with an upper limit of growth 2-3 times as deep. The index is more comparable to that in a medium containing

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growth. The index is obtained by dividing average of sum of upper limits of growth by average of sum of widths of growth.

Although depths and widths in A, D, E vary indexes are similar indicating a logarithmic relationship. Reason for various changes will be apparent from text. Increased width of B over A is recorded purposely since there are variations with runs. The variations are of the same order and do not usually confuse results. Good growths must be selected for observations since sparse growths will be deeper. Smaller variations observed on addition of non-reducing salts to mediums have been omitted because minor variables exist making them uninterpretable.

0.5 gms. NB $\left(\frac{7.87}{9.93}=0.778\right)$ instead of 2 gms. NB. As will be explained later, two changes dependent within limits on the extent and intensity of growth can be measured below the initial growth at atmospheric pressure, a decreased pH and lowered Eh. These changes are obviously not sufficient to markedly affect growth. Nor does the reduction effect exert itself like reducing agents such as cysteine and sodium lactate (5 gms./1,000 cc. added to medium containing 2 gms. NB) which narrow the spectrum and decrease the depth of the upper delimitation of growth. Similarly, less dense, haze like, poorly delimited growths seem to allow more ready diffusion of oxygen with a resulting more diffuse and generalized growth. In the diffuse growth produced when dextrose (1 gm./1,000 cc.) is added to the medium another factor may play a role—its greater energy afforded for anaerobic growth. Increased temperature within limits may play a role similar to dextrose.

When nutrient is markedly decreased (0.5 gms. NB) the growth was less dense but delimitations of bands were well maintained. The upper limit of growth was proportionately deeper and as the spectrum widened bands appeared often with clear spaces between them; often with time bands were added. There was a decrease in density of growth of organisms with decrease in nutrient beyond a certain point (2 gms. NB and less). The almost logarithmic increase in width of growth with increase in depth of upper delimitation with other findings suggested a gradient of diffusion of oxygen in these mediums. This is extremely hard to prove. A technique employed consisted in sealing a $\frac{1}{2}''$ x 4.5″ test tube whose bottom was pinholed and covered with $\frac{1}{2}''$ plaster of paris into a 3/4″ x 5.5″ test tube, plugging, sterilizing, pouring inoculated medium of 1 into inner test tube and incubating 48 hrs. at 60 lbs. O_2. Since medium intervened between the atmospheric pressure between the inner and outer test tube, increased pressure on the outside resulted in equilization of oxygen tension through the medium. Results were somewhat masqued since some medium was forced through plaster of paris and pinholes; nevertheless, a band of growth resulted 4-5 times as deep as the control. While this is suggestive much more involved and accurate work and a seal which will prevent passage of medium but allow passage of O_2 are needed.

When mediums (2 gms. NB) upon which organisms have grown 48

hrs. were autoclaved, replanted and reincubated under increased tension growths suggesting decreased nutrient were obtained. Similar results were obtained when filtrates of 48 hr. broth cultures of 1 containing originally 2 gms. NB were made into solid shakes by addition of 7.5 gms. N B/1,000 cc., replanted and reincubated for 48 hrs. under 60 lbs. oxygen.*

Number of organisms planted or number of organisms establishing growth especially if growth was established as granular colonies modifies depth of growth. As colonies become more discrete and spectrums less dense, width and depth of growth became greater. Granules tend to be larger and growth denser just below the upper delimitation and size of granules tends to decrease with distance from this line. Often a decrease in size of granules almost to the bottom of the tube was apparent; the number of organisms establishing growth in unit area is not necessarily less. Type of granules and their distribution varied with the organism. Not only does decrease in number of organisms planted produce first fine granularity, then coarse granularity with finally discrete colonies here and there, but also substances (sodium chloride, potassium chloride, sulfanilamide, potassium cyanide, etc.) tested in increasing concentration. The latter indicates an increasing inhibiting effect preventing some organisms from establishing growth. Certain organisms are affected first. A more diffuse distribution of granules corresponds to masquing of delimitations and a distribution of haze of growth in the medium. Haziness may be indicative of inhibition by certain substances; for example, increase in acidity or alkalinity seems to show such inhibition of growth which is concentrated more at one area, the apparent point of departure of growth. Haziness indicates a decreased growth of organisms, while discrete colonies a prevention of growth by inhibiting agents of less resistant organisms. The more general distribution with possibly greater diffusion of oxygen due to utilization of factors inhibiting permeating of oxygen apparently holds in either instance.

As stated before, reducing substances such as sodium lactate decrease the upper delimitation of growth and almost logarithmically narrow the growth.

The only hydrogen acceptor tested was methylene blue (3

*I wish to thank Miss Jean Blaustein for technical assistance in this phase of the work.

mgm./1,000 cc.). It acted with certain organismal systems and increased the depths of 2, 3, 9, 13, 21. The entire increase of depth may not always be due to methylene blue since the dye inhibits certain growths.

When nutrient is much diluted plain agar shakes must be run since agar contains traces of nutrient sufficient for slight growth of certain organisms. The proportionate depth of organisms must indicate their relative aerobic or anaerobic nature. Some aerobes mixed with anaerobes (Cl. sporogenes) apparently sufficiently reduce medium below to allow growth of the anaerobe. When two aerobes are mixed and grown there is a tendency for the spectrum of one to predominate. Some organisms apparently grow under stress, since when various levels of the spectrum of 1 are stained and examined microscopically organisms at the lower levels may show bizarre shapes while those above appear normal. Variations noted should reflect themselves under like conditions in aerobic growth. The atmosphere-medium relationship and other changes (Williams, c, d, e) studied in pathogenic fungi apply to a variable degree in bacteria. The probable surface tension effect on pathogenic fungi has not been identified in bacterial growth spectrums. Possible oxygen tension variants in the separated bands of spectrums remain to be proven. A wider spectrum probably indicates a wider oxygen tension range of the specific organism. Assuming a gradient of oxygen diffusion, depth of growth range may be related to the demand of oxygen in unit time. Thus establishment of growth slowly or by fewer organisms would demand less oxygen in unit time and the range of growth would be lower in the medium. Such reasoning might at least in part explain increase depth comparable to growth on dilution of nutrient and reduction of number of organisms establishing growth.

Width of growth. Width of growth is dependent on time of growth, percentage of nutrient, oxygen-carbon dioxide ratio, growth inhibiting factors, reducing and oxidizing agents, number of organisms planted, temperature of growth and its initiation, substances encouraging anaerobic growth. This problem has been amply discussed under depth of growth.

Consistency of growth. This depends on the same factors as width of growth and has been amply discussed under depth of growth.

Effect of growth under 60 *lbs. oxygen on adjacent medium.* Tech-

nique: Alcoholic solution of bromthymol blue (0.08 gms.) was added to medium containing 2 gms. NB to observe acidity change and 3 mgm. methylene blue to observe concentration and reduction of dye. The lower part of test tubes (medium 55-60 mm. deep) were scratched around with a file, encircled with resistance wire and an electric current applied until cracked. Medium was slid out, cut at various depths and Eh and pH taken with the Beckman potentiometer. Organisms were observed for effects on dyes and controls, 1, 2, 3, 5, 6, 7, for pH and Eh (readings in volts with KCl—Calomel as reference electrode).

Acidity changes in dye were absent from above the growth but usually present in and below the growth for 30 mm. or more. Control medium showed a pH variation of 7.35-7.5 at various depths, while mediums with growth often showed a slightly more alkaline or similar surface reaction, an area of growth usually 0.3 to 0.7 more acid, depending on the depth in the growth and the organism, more acid with increasing depth below the growth (as great as pH 6.2 with 8) up to 30 to 40 mm. where a reversal to a more alkaline reaction usually occurred. When 5 gm. monobasic sodium phosphate/1,000 cc. (showed similar type growths to controls) was included in medium changes in pH of 8 and 1 were much less but when 1 gm. dextrose/1,000 cc. was included much greater acidity changes resulted (as great as pH 4.27 with 1). Change varied with organisms, such organisms as 2 producing little change. Organisms grown in atmosphere (here growth is within 2 mm. of the surface) showed changes similar to those for growths under increased pressure.

A pH range of medium from 5.85 to 8.2 had little effect on the width and depth of growth. Such a range resulted in a surface Eh from + .27 to + .13v. Beyond these ranges of pH inhibition of growth occurred first for certain organisms then for others (Williams, 1939).

Reduction of methylene blue showed a correspondence to Eh variation. Unreduced dye was present above the growth and in some instances extended for 2-10 mm. below the growth. Dye below the growth tended to disappear on standing. Some growths showed reduction immediately, some on standing and some reduction only at their lower edge. All growth spectrums showed varying intensity of bands and lines of dye concentration after standing or even before. Colors of concentration were green, violet, purple, blue to almost black,

PHOTOGRAPHS ILLUSTRATING VARIATIONS IN GROWTH SPECTRUMS

By turning a film 1½ inch each time in a folding camera provided with a special lens a 1:1 photograph of each shake growth is recorded. The wide dark line to the right of the number indicates the upper level and the meniscus of the medium: as we look further to the right we look deeper into the medium. The photographs illustrate many of the fundamentals discussed in the text. Time of incubation was 48 hrs. and pressure 60 lbs. oxygen except where specified.

254 *S. enteritidis.* 8gms. NB, 7.5 gms. agar/1,000cc.
284 *S. enteritidis.* 4 gms. NB, 7.5 gms. agar/1,000cc. Wider and deeper.
91 *S. enteritidis.* 2gms. NB, 7.5 gms. agar/1,000cc. Bands, wider, deeper.
179 *B. anthracis.* 2gms. NB, 5gms. cysteine HCl, 7.5gms. agar/1,000cc. Narrower and less depth than 50.
50 *B. anthracis.* 2gms. NB, 7.5gms. agar/1,000cc.
49 *B. anthracis.* 2gms. NB, 3 1/3mgm. methyiene blue, 7.5gm. agar/1,000cc. Increased depth over 50.

indicating a variable degree of oxidation of methylene blue to azures. Frequent delay in appearance of lines of dye concentration suggested a change from the leuco to the blue dye occured after metabolism was reduced. Intensity of blue below the growths was markedly reduced; reduction was not always complete to the bottom of the tube. With exception of 1 where there was marked transition from reduced (at termination of the cloud of growth below spectrum) to oxidized dye the change (usually at 40-50 mm. in medium of 60 mm. deep tubes) in bottom of tubes was more gradual. Several lines of dye concentration frequently of different color occurred with organisms when nutrient was dilute and spectrum wide. Deeply stained organisms often were seen microscopically in lines of dye concentration. Trypan blue (15 mgm./1,000cc.) (an acid dye) was concentrated only by a limited number of organisms.

Because of drift (as much as .03v.) $\frac{1}{2}$, 1 and $1\frac{1}{2}$ min. Eh readings

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2 *E. coli communis.* 2gms. NB, 7.5gms. agar/1,000cc.

303 *Ps. aeruginosa.* 2gms. NB, 3. 1/3 mgm. methylene. blue, 7.5gms. agar/1,000cc.

351 *Ps. aeruginosa.* 0.5gms. NB, 3. 1/3 mgm. methylene blue, 7.5gms. agar/1,000cc. Widening, lowering and separate bands.

NEAREST APPROACH TO CELLULAR EFFECT.

45 *Staph. albus.* 2gms. NB, 5mgm. methylene blue, 7.5gms. agar/1,000cc.

SWIRLS

al**33** *Ps. fluorescens.* 2gms. NB, 50gms. NaCl, 7.5gms. agar/1,000cc. 30 lbs. O_2. 72 hrs. incubation.

106*B Proteus vulgaris.* 2gms. NB, 7.5gms. agar/1,000cc. 120 lbs. O_2, 20 lbs. CO_2.

DISCRETE

48*B Staph. aureus.* 2gms. NB, 1gm. dextrose, 7.5gms. agar/1,000cc. Reduced number of organisms planted.

CONTINUOUS HAZE

108 *B. megatherium.* 2gms. NB, 7.5gms. agar/1,000cc. 24 hrs. incubation.

BACTERIAL GROWTHS RESEMBLING PRECIPITATIONS IN GELS.

LIESEGANG RINGS

57 *Proteus* X-19. 2gms. NB, 5mgm. methylene blue, 7.5 gms. agar/1,000cc. Methylene blue effect comparable to decreased nutrient.

772 *B. mycoides.* 1gm. NB, 7.5gms. agar/1,000cc. 72 hrs. incubation.

845 *Staph. aureus and B. subtilis* in same medium. 2gms. NB, 7.5 agar/1,000cc. 110 lbs. O_2. 96 hrs. incubation.

56 *S. enteritidis.* 0.5gms. NB, 7.5gms. agar/1,000cc.

60 *Proteus* X-19. 0.5gms. NB, 7.5gms. agar/1,000cc.

NOTE Photographs are given of depth of *B. anthracis* being increased by methylene blue yet it is not sited in the text as one of the organisms whose depth is increased by methylene blue. This is because methylene blue has a variable effect as yet uninterpretable on this organism. In our experience this variability is not the rule.

were taken. It was necessary to insert electrodes several millimeters so that readings are actually over this depth; 1 min. readings were used in interpretations. The drifts were consistent in one or other direction and did not modify interpretations. Controls varied from + .148 to + .16v. Surfaces of shakes were + .159 to + .02, growth areas .02 to .09 more reduced, with gradual increase in reduction (extreme − .225 with 8) for 10-40 mm. where there was often a gradual reversal to a more oxidized state. Dextrose (1 gm./1,000cc.) markedly increased reduction effect (− .515 with 8). Tubes grown in atmosphere showed variation of order of changes for growths in shakes grown under pressure.

Correlations in colloidal chemistry. By including a dilute solution of one reactant in a gel, and layering a more concentrated solution of the other reactant on the hardened gel five types of structure, viz. continuous, discrete, cellular, periodic and swirls of precipitate have been observed. In this work (see Plate) bacterial growths resembling a haze of growth, discrete colonies, periodic structures (Liesegang rings), swirls of precipitate and floccules of growth have been observed.

Thus, the bacterial medium represents one reactant and oxygen the other with living organism, the "catalyst", resulting in more organisms comparable to precipitates of colloidal chemistry. Only theories (Hedges, 1931) exist in explanation of precipitates in colloidal chemistry. Organismal bands of growth may reduce and deplete the area below rendering it temporarily uninhabitable but coincidently use up and oxidize material active in preventing diffusions of oxygen so that when the effect is decreased sufficiently growth is established as the next band. Precipitates show a tendency to be progressively farther apart with depth in gel while bacteria growing in rings show variation.

SUMMARY

Depth is upper limit of spectral growths varies with time of growth, oxygen tension, carbon dioxide tension, temperature of incubation, number of organisms planted, amount of nutrient, growth inhibiting factors, reducing and oxidizing agents.

Width of spectral growth varies with time of growth, percentage of nutrient, growth inhibiting factors, reducing and oxidizing agents, organisms establishing growth, temperature of growth, substances encouraging anaerobic growth.

Consistency of spectrum varies with time of growth, percentage of

nutrient, growth inhibiting factors, reducing and oxidizing agents, organisms establishing growth, temperature of growth, substances encouraging anaerobic growth.

Within limits oxygen counteracts inhibiting effects of carbon dioxide.

Eh and pH of medium above spectrum are similar to controls but decrease usually occurs in and below the growth to a varying extent and depth with certain organisms. Changes in bromthymol blue and methylene blue included in mediums are what would be expected from Eh and pH readings.

All organisms studied are capable of concentrating methylene blue to a variable extent in lines and bands in and directly above their spectrums and some are able to change the blue to its various azures.

Explanation of the various changes is suggested. Suggestive evidence is presented indicating a gradient of oxygen diffusion in mediums studied.

Most appearences produced with inanimate precipitates in gels have been produced by bacterial growths in shake cultures.

It is felt that the great possibilities presented by growing microorganisms under increased gas tensions should win this method a place with anaerobic and aerobic methods of growth in bacteriological investigations.

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l-LEUCINE AND DEVELOPMENTAL GROWTH

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INTRODUCTION

Much work with leucine is reported. Its significance is clouded by contradictions. These perhaps derive from the use of impure products and ignorance of their toxicity in low concentrations. There are reports of toxicity in high concentrations (King '12; Burrows and Neymann '17; Gordon and M'Leod '26).

As far as growth is concerned most writers record either no action or inhibition (Petrov '13; Burrows and Neymann '17; Davis and Ferry '19; Gordon and M'Leod '26; Holt '28; Vles and de Coulon '30; Odiette '32; Odiette and Truhaut '33; Sagastume and Solari '33; Suzuki and Miyao '33; Ehrismann and Dramburg '37; Ark '37). Only Fildes and Richardson ('35) and Womack and Rose ('36) indicate the amino acid is important in this respect.

Its nitrogen is utilized by plants other than bacteria as it is by animals (Molliard '10; Petrov '13) and it has been found non-toxic thereto (Shreiner and Reed '08).

In view of these earlier findings our results will have a peculiar interest since they were obtained by the use of as pure a l-leucine as could be gotten from natural sources and explorations were made in non-toxic concentrations.

PROCEDURE

Experiments were run in 1933 and 1938 with two different sets of workers. There were 78 trials and close to 42,000 animals (*Obelia geniculata*) half of which were exposed to l-leucine in concentrations from M/200,000 to M/3400. Test and control cultures were run simultaneously under like conditions of temperature, illumination and pH. Four changes into fresh solutions were made during the 24 hour course of each experiment. The data are in table 1 which is derived, constructed, and analyzed as described in an earlier report (Hammett and Schlumberger '37).

The 1933 experiments were run before recognition that methionine is a common contaminant of leucine. They were few and done at toxic concentrations. Analysis of the growth reaction is therefore restricted to the 1938 series. This was run with l-leucine prepared by Dr. M. S. Dunn of the Amino Acid Manufactures, University of California, Los Angeles. The product was in two lots. One was reported to have no more than 0.004 per cent sulfur; no more than 0.004 per cent Cl^-, NH_3^+, P_2O_5, and heavy metals; and a rotation of $[\alpha]_d^{26}$—10.6°±0.05° Aq.. The other was reported to be negative for NH_3^+, $Fe,^{+++}$ and heavy metals; to have less than 0.016 per cent sulfur; and a rotation of $[\alpha]_d^{28}$—10.0°±0.5° Aq.

TABLE I

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN l-LEUCINE CULTURES. ALSO THE NUMBER OF EXPERIMENTS, COLONIES, HYDRANTHS, AND GONOPHORES AT THE STATED CONCENTRATIONS.

	1938														1933*			
M/1000	200		100		50		25		12.5		50–16.7		10–6.8		5–3.4	
No. Expt	12		12		12		12		12		3		5		10	
	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test
Cols	180	180	180	180	180	180	180	180	180	180	38	38	64	64	124	124
Hyds	3339	3383	3475	3473	3600	3597	3805	3818	3480	3492	524	516	873	862	1759	1746
Gons	1074	1078	883	877	773	712	359	357	425	435	47	34	89	69	172	140
	GROWTH															
	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=
Init	3	3	1	4	2	1	2	3	2	0	3	4	1	4	2	4	3	0	1	4	2	2	4	1
Prol	6	1	1	4	1	3	1	3	4	3	2	3	4	1	3	0	2	3	1	4	2	2	4	2
Diff	1	3	4	3	3	2	3	0	5	3	2	4	4	3	2	3	0	4	3	1	5	1	4	5
Orgn	2	2	4	2	2	2	3	0	4	2	4	2	0	4	4	2	2	3	3	2	2	2	3	3
	METABOLISM (By Single Experiment)															
Main	5	6	1	5	6	1	5	6	1	5	4	3	10	1	1	2	1	0	2	3	0	0	9	1
Catb	6	4	2	3	6	3	7	5	0	6	6	0	4	7	1	1	2	0	1	3	1	1	9	0
	METABOLISM (By Combined Experiment)															
	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test
Regr	18.3	18.9	20.5	21.6	22.3	24.6	22.6	22.2	37.5	34.6	31.2	27.6	34.3	38.4	33.1	40.0
Catb	65.2	66.7	67.3	64.0	58.7	55.0	66.8	65.0	62.6	59.9	88.1	84.6	91.7	88.3	94.9	90.4
	Increase in Percentage Regression															
%	3.3		5.4		10.3		−1.8		−7.7		−11.5		11.9		20.8	

*One experiment at M/50,000: one at M/25,000: one at M/16,700: one at M/10,000: four at M/6800: four at M/5000: three at M/4000: and three at M/3400.

The first lot was used first because of its low sulfur content. It sufficed for all the experiments done at M/200,000, M/100,000, M/50,000 and M/25,000. It was necessary to use the second lot with its relatively high sulfur content in the M/12,500 series. But here no more than 4 of the 0.066 gram samples were taken.

RESULTS

l-Leucine was toxic to obelia at M/25,000 and upwards. Thus—whereas at M/50,000 test growth was greater than control in 2 of the 4 developmental activities and less in 1, at M/25,000 it was less in 2 and greater in none. Further—of the 34 possible growth expressions at M/50,000, 9 were greater in test than control and 6 were less, while at M/25,000 11 were less and 8 were greater. A shift towards change in direction of catabolic activity and regression is consistent (Hammett '36; Hammett and Toman '39).

Analysis of growth reaction is therefore made from the M/200,000-M/100,000-M/50,000 data.

Initiation was unaffected as a developmental activity. Test was greater than control in 9, less in 8, and equal in 4 of the 21 expressions given by the 3 sets of experiments. By concentrations it was greater in 1 and equal in 2. And when the data are combined it is greater in 2, less in 3 and equal in 2 of the 7 potentialities for activity expression.

Stolon growth was persistently enhanced. This extended into the toxic range. The expression was greater in 5 and less in none of the 8 concentration series, and more stolons were produced in 33 of the 78 experiments and less in but 18.

Hydranth proliferation was definitely enhanced. Test was greater than control in 10, and less in but 3 of the 18 expressions given by the 3 sets of experiments; by concentrations it was greater in 2 and less in 1; and when the data are combined it is greater in 5 and less in none of the 6 potentialities for activity expression. Gonophore reaction was indecisive.

Differentiation was unaffected as a developmental activity. Test growth was greater than control in 8, less in 7, and equal in 12 of the 27 expressions given by the 3 sets of experiments; by concentrations it was greater in 1, less in 1, and equal in 1; and when the data are combined it is greater in 2, less in 2, and equal in 4 of the 10 potentialities, two of which were unexpressed.

The developmental activity of organization tended to be better than usual in the presence of l-leucine. Test was greater than control in 7 and less in but 4 of the 21 expressions given by the 3 sets of experiments; by concentrations it was better in 1 and less in none; and when the data are combined it is better in 2, less in none, and equal in 6 of the 9 potentialities one of which was not expressed.

By single experiment maintenance was hardly affected though hint of a cut is given. When the data are combined however the percentage regression in the tests is seen to be greater than in the controls. This difference increases with increase in concentration up to the toxic level. This is evidence of specific dynamic action (Hammett '36a). At M/25,000 maintenance is sustained and regression reduced. At the higher more toxic concentrations regression is again increased with increase in concentration.

There is no evidence that catabolic disintegration of the senile hydranths was affected consistently one way or the other in the non- toxic range. In the toxic range it was reduced as has been found previously.

DISCUSSION

Proliferation was enhanced in the presence of l-leucine. SH is a specific stimulus to cell increase in number (Hammett '29). Leucine is commonly contaminated with methionine which is a potential source of SH (Mueller '35: du Vigneaud, Dyer and Harmon '33: Hammett and Padis '36).

The question obtrudes—is the proliferation reaction to be attributed to l-leucine or to a contamination thereof with SH-producing methionine?

Extreme accuracy of sulfur determination in organic material is difficult to attain. Particularly when minute amounts are involved. It is wise to grant an error of at least 200 per cent. Assuming that there was three times as much sulfur in the low sulfur leucine sample as reported, the following amounts might have been present in the cultures as added by contamination: 8×10^{-11} in the M/200,000; 1.6×10^{-10} in the M/100,000; and 3.2×10^{-10} in the M/50,000. In no case have concentrations of S as SH as low as these been found to forward cell increase in number. Ten times as much as the highest amount here represented is the usual lower limit of effectiveness (Hammett '29). The concentrations in the toxic range would not have exceeded 6.4×10^{-10} for the M/25,000 series, and 1.2×10^{-9} in those M/12,500 cultures in which this lot of l-leucine was used. In those in which the high sulfur lot was used the possible concentration ranged from 8×10^{-10} to 3×10^{-9}.

It therefore does not seem as if sufficient methionine could have been added along with the l-leucine to have by itself produced the pro-

liferation reaction. Nor does it seem possible that it sufficiently increased the culture content to bring about the change.

Two possibilities remain. Either l-leucine alone is a determining participant in some process essential to cell increase in number, or it facilitates the proliferation-forwarding SH group of methionine.

Since there are many separate but associated sets of processes concerned in cell division, and since each has its own chemical participants and determinants, it is possible that l-leucine is a specific chemical factor in one of them.

On the other hand the persistence of physical association of methionine with leucine is noteworthy. It may be that this is simply a result of possession by both of a physical or chemical property which leads to a conjoined separating out under the conditions ordinarily used for leucine isolation. But it may also mean that there is a functional association. Or even that leucine is a precursor of methionine.

It thus may be that l-leucine acts either to facilitate the production of the proliferation-forwarding SH from methionine, or to facilitate the action of this group on cell division. If this be so the enhanced proliferation is not due to a direct action of l-leucine on any process concerned therein, but to an indirect effect exerted on methionine.

Even if l-leucine by itself is found to be a direct participant in some process specific to cell division it still can be considered as functionally related to methionine since this too through its SH-producing potentiality is concerned in the activity (Chase and Lewis '33; Hammett and Padis '36; Hammett '29) and all the processes of cell division are indubitably interrelated.

Which if any of these possibilities is the fact cannot be determined from present data.

In 1936 Womack and Rose presented a report on "The Relation of Leucine, Isoleucine, and Norleucine to Growth". The results show beyond question that dl-leucine and dl-isoleucine are indispensable dietary components. It may be questioned whether they show any specific or direct relation of these amino acids to growth.

Their omission from the diet of rats entails loss of weight. Failure to gain weight under such conditions might possibly be admitted as evidence a growth factor is concerned; but failure to maintain weight is evidence some other activity than growth is sustained.

When the amino acids were admitted separately to the diet or in combination with dl-norleucine no weight was gained. Weight was lost. This loss of weight was greater than that which occurred when the amino acids were omitted from the diet entirely. Thus weight loss in two sets of rats without leucine and isoleucine averaged 0.32 and 0.56 gram per day per rat, while the weight loss in two sets of rats having either leucine or isoleucine alone or in combination with norleucine averaged 0.64 and 0.65 gram per rat per day. This might be taken as evidence of toxic action.

On the other hand two sets of rats which had both dl-leucine and dl-isoleucine in the diet with and without dl-norleucine gained the to-be-expected weight (Donaldson '24). Perhaps the explanation lies in the fact that when the compounds were fed singly the amount of food eaten was but 47.0 and 48.7 grams on the average; while when they were fed together over like periods the food eaten averaged 187.0 and 156.2 grams respectively.

The rats fed dl-leucine and dl-isoleucine together ate from three to four times as much food as those fed these amino acids separately. This might eliminate toxicity as a factor.

It would seem as if the gain instead of loss in weight should be attributed to increased food consumption rather than to any direct or specific effect of dl-leucine and dl-isoleucine on growth.

The basis for a claim that these compounds have a direct relation to growth seems hardly established by these data. And of course they give no evidence, nor can they, that leucine alone is a factor (Hammett '36b).

The data do suggest however that the two amino acids may constitute a specific appetite factor. Their presence in the diet might thus be necessary for adequate nutrition.

That l-leucine is a stimulant of metabolic activity with probably some specific dynamic action is indicated by the obelia reaction. This is consistent with the observations of Herzog and Saladin '11; Rapport and Beard '27; Ort and Bollman '27; and Kisch '32. The effect however was slight and this in turn is more or less consistent with the findings of Lusk '12; Seth and Luck '25; Anderson and Emmert '34; and Miyao '35. This may or may not be associated with its deamination proclivities (Snyder and Corley '38; Mitchell and Hamilton '29)

The obelia regression reaction to l-leucine was less than that to d-isoleucine. Using only data from assuredly non-toxic concentrations it is seen that test regression was increased over control in 79 per cent of the d-isoleucine experiments which deviated, while in the l-leucine series increase occurred in but 55. Further,—the average regression increase due to d-isoleucine was 10.9 per cent while that due to l-leucine was 6.3.

Despite this reduction in intensity of influence which accompanied the structural change,—l-leucine with its added CH_2 was yet a greater stimulus to regression than d-valine with its one less CH_2. Using the same data the drift is hinted at in the single experiment record. Here regression was increased over control in 53 per cent of the d-valine experiments which deviated,—and in 55 per cent of the l-leucine. The trend is established by the average regression increase where that due to l-leucine was 6.3 per cent, and that due to d-valine was but 2.2. This sustains and strengthens the conclusion that the addition of CH_2 to the molecule of these naturally occurring tissue components of general distribution determines an increased regression (Hammett and Toman '39). It may be interpreted to mean that the added group is a stimulus to metabolic activity.

Though l-leucine has effects which are produced by other amino acids the constellation of influence on obelia is unique to the compound. This gives one more piece of evidence that each of these naturally occurring substances has its own individual role to play in the growth picture.

SUMMARY AND CONCLUSION

Seventy-eight experiments with close to 42,000 animals (*Obelia geniculata*) half of which were exposed to l-leucine in concentrations ranging from M/200,000 to M/3400 in two seasons with two sets of workers showed that the compound:—

1. Is toxic at M/25,000 and over.
2. Has no effect on the developmental activities of initiation and differentiation.
3. Enhances hydranth proliferation expression.
4. Enhances stolon production.
5. Tends to enhance organization expression.
6. Cuts maintenance and increases regression slightly.
7. Fails to affect catabolic activity.

The possibility that the proliferation reaction may be due to contamination with SH is discussed.

The compound is less stimulative of regression than d-isoleucine. It is more stimulative than d-valine. This sustains the contention that added CH_2 is determinative of metabolic increase.

The work was done at the Marine Experimental Station of The Lankenau Hospital Research Institute with aid from Miss A. Bein of Philadelphia, and the technical assistance of Misses Chatalbash, Elliott, Evangelides, Steele, Hyde, and Comee, and Messers Rivard, Toman, and Chapman.

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d-ISOLEUCINE IN DEVELOPMENTAL GROWTH

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INTRODUCTION

The literature on d-isoleucine is sparse and unsatisfactory. Much seems to be taken for granted. But little critical examination of constitution and distribution is had. Surety as to which isomer is actually a constituent of protein is lacking and the distribution and amount of this amino acid in protein material is uncertain because its percentage is reported along with that of leucine instead of by itself (Mitchell and Hamilton '29).

This being the case the reports that isoleucine may be replaced in the diet by leucine (Mitchell and Hamilton '29); that the amino acid is without effect in giant yeast cell production (Holt '28); that it has less influence than leucine on tissue respiration (Kisch '32); and that it causes crown galls to gradually collapse and dry up (Ark '37) are possibly somewhat less useful than interesting.

Clarification is however beginning for Snyder and Corley ('38) are studying the influence of structural configuration in the alpha amino acids on their deamination in the dog. Of present interest is the report that l(+)-isoleucine and l(+)-alloisoleucine are readily deaminated after subcutaneous injection while d(−)-isoleucine is not. This difference alone is evidence of the need for exact definition in these matters.

The state of knowledge being what it is all that could be done in these growth studies was to use an isoleucine having as close as possible the physical characteristics pertaining to the naturally occurring (sic) product as recorded in the literature.

In 1933 the best material obtainable at that time was used. But it was far from satisfactory. So in 1938 a better product was made by Dr. T. F. Lavine of The Institute Staff. This lot after repeated recrystallization had a rotation of $[\alpha]_d^{20}+39.6$ and was 99.74-100.15 per cent pure d-isoleucine according to titration with sodium methylate. Sulfur was undetectable and heavy metals by dithizone were indeterminate.

A total of 80 experiments were run during 1933 and 1938 with two different sets of workers and close to 38,000 animals. Stock solutions containing 0.066 gram per 100cc fresh sea water provided daily the aliquots from which the test culture solutions were made. The colonial-living form of the marine hydroid *Obelia geniculata* was the test animal. Like number of test and control animals of like distribution according to state of development at the beginning of observation were simultaneously grown in fresh sea water cultures under like conditions of temperature, illumination and pH. Four changes into fresh solutions were made during the 24 hour course of experiment. The amino acid concentration ranged from M/400,000 to M/3400.

The usual data in the usual form are given in Table 1. They are derived and analyzed as described in detail in the l-aspartic acid report (Hammett and Schlumberger '37).

TABLE I

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN D-ISOLEUCINE CULTURES. ALSO THE NUMBER OF COLONIES, HYDRANTHS, AND GONOPHORES AT THE STATED CONCENTRATIONS

	GROWTH										NUMBER					
	INIT			PROL			DIFF			ORGN			Expt	Colonies		Hydranths		Gonophs	
M/1000X	+	−	=	+	−	=	+	−	=	+	−	=		Cont	Test	Cont	Test	Cont	Test
400	1	1	5	3	5	0	3	1	4	4	2	2	10	150	150	2641	2635	343	337
200	1	4	2	3	3	2	3	3	4	1	2	5	10	142	142	2745	2751	251	356
100	1	4	2	5	1	2	3	4	2	2	2	4	12	180	180	3522	3524	288	305
50	0	5	2	2	4	2	2	6	2	5	0	4	10	150	150	2612	2599	288	299
25	2	2	3	2	4	2	3	4	3	3	1	4	10	150	150	2670	2673	196	204
12.5	3	2	2	4	3	1	2	7	1	1	4	4	10	149	149	2667	2667	214	283
25–12.5*	1	4	2	2	1	4	2	4	3	0	2	4	2	24	24	373	372	19	7
8.3–6.7*	3	3	1	3	2	3	4	2	4	1	4	3	8	96	96	1411	1405	120	67
5.0–3.4*	4	0	3	2	3	3	6	4	0	2	2	4	8	96	96	1440	1445	159	128

	METABOLISM												
	MAIN			REGR			CATB			% Main		% Catab.		% Regress.		% Inc.
M/1000X	+	−	=	+	−	=	+	−	=	Cont	Test	Cont	Test	Cont	Test	Regress.
400	5	4	1	4	5	1	6	4	0	69.9	70.3	76.7	76.4	30.1	29.7	−1.3
200	2	6	2	6	2	2	4	5	1	70.8	68.4	56.0	54.4	29.2	31.6	8.2
100	2	9	1	9	2	1	6	4	2	80.3	77.6	66.2	67.2	19.7	22.4	13.7
50	3	6	1	6	3	1	3	7	0	74.2	72.6	77.7	70.8	25.8	27.4	6.2
25	5	5	0	5	5	0	3	6	1	69.6	70.0	67.1	60.6	30.4	30.0	−1.4
12.5	4	5	1	5	4	1	4	6	0	66.7	67.6	76.3	76.0	33.3	32.4	−2.7
25–12.5*	2	0	0	0	2	0	1	1	0	57.5	61.3	97.2	90.3	42.5	38.7	−9.0
8.3–6.7*	2	5	1	5	1	2	1	7	0	64.6	64.2	92.9	86.1	35.4	35.7	0.8
5.0–3.4*	4	4	0	4	4	0	4	4	0	53.7	51.8	88.9	88.1	46.3	48.2	4.1

*1933 Results. All others 1938 One trial at M/25,000: one at M/12,500: 4 at M/8300: 4 at M/6700: one at M/5000: 4 at M/4000: 3 at M/3400.

RESULTS

Toxicity appears at M/50,000. The evidence;—whereas 12 of the 34 growth expressions were greater in test than control at M/100,000 and 11 were less, at M/50,000 15 were less and but 9 were greater:—at

M/100,000 test growth was greater than control in 1 and less in 1 of the four developmental activities, while at M/50,000 it was less in 3 and greater in but 1. Consistent is the shift to lessened regression differential and the shift in catabolic activity from a state of slight enhancement to one of decided inhibition (Hammett '36; '36a).

Above M/12,500 however the toxic effect seems to disappear and to be replaced at the highest level by a non-specific better-than-usual growth. Thus whereas test growth was less than control in 16 and greater in but 10 of the 34 expressions at M/12,500 (1938 data; similar in the 1933), at M/8300-6700 the differential disappeared to be reversed at M/5000-3400 with test growth being greater in 14 and less in but 9. Further--test developmental activity was less than control in 2 of the 4 brackets at M/12,500 (1938 data; similar in the 1933); at the M/8300-6700 range the differential disappeared; at the M/5000-3400 range it was reversed with test activity greater than control in 2 and less in none. This phenomenon though rare has appeared elsewhere (Hammett '37).

Analysis of growth reaction is made from the M/200,000 and M/100,000 data since experiments done at lower concentration show nothing consistent and those done at higher give evidence of toxicity.

Initiation is retarded in the presence of d-isoleucine. The evidence;—test expression is less than control in 8 and greater in but 2 of the 14 expressions afforded by the 2 sets of experiments; it is less in both concentrations; and when the data are combined it is less in 4 and greater in none of the 7 potentialities for developmental activity.

Proliferation may have been favored. Thus—test was greater than control in 8 and less in but 1 of the 16 expressions given by the 2 sets of experiments; it was greater in 1 and less in none of the 2 concentrations; and when the data are combined it was greater in 4 and less in but 2 of the 8 potentialities for developmental activity. A hint of similar trend is given by the 1933 data in the lower concentrations.

Differentiation was unaffected. Thus—test was greater than control in 6 and less in 7 of the 20 expressions given by the 2 sets of experiments; it did not differ from control in either concentration; and when the data are combined it was greater in 2, less in 2, and equal in 6 of the 10 potentialities for developmental activity.

Organization also was essentially unaffected. Thus—although test was less than control in 5 and greater in but 3 of the 18 expressions

given by the 2 sets of experiments, it did not differ from control to significant extent in either concentration, and when the data are combined it was greater than control in 3, less in 2, and equal in 3 of the 9 potentialities for developmental activity.

Despite these developmental activity apathies it is possible that *hydranth* growth was favored by d-isoleucine. Thus, hydranth proliferation expression was greater in the tests in 6 and less in but 3 of the 12 expressions given by the 2 sets of experiments; differentiation was greater in 5 and less in 4; and organization was greater in 3 and less in none. Gonophore reaction was inconsistent.

Maintenance tended to be depressed and regression increased in the non-toxic effective range. The fact that the reaction increased from 8.2% to 13.7% with increase in concentration from M/200,000 to M/100,000 and then decreased to 6.2% with further increase in concentration to M/50,000 suggests a specific dynamic effect (Hammett '36). The inhibition of regression in the toxic M/25,000-12,000 range was reversed at the higher apparently non-toxic levels. This has occurred elsewhere (Hammett '37a).

Catabolic disintegration of the senile animals may have been held back slightly. The tendency is seen in 5 of the 9 sets of experiments. The opposite trend occurred in but 2.

DISCUSSION

The outstanding growth reaction to d-isoleucine is inhibition of new structure production. This means that the compound participates in some process specific to initiation. Anlagen activity seems to be most affected. Apparently a primary site of action of the amino acid is on some process concerned in this particular type of tissue organization for new structure emergence. It follows that the retardation of initiation expression from other sources is probably factored by the anlagen processes which participate therein. This is not impossible since initiation is compounded of many sets of processes to which some peculiar to anlagen activity undoubtedly contribute (Hammett '36b).

It should be noted that the slight trend to enhancement of proliferation expression might have derived from contamination of the d-isoleucine with some naturally occurring sulfur-carrying compound such as methionine which would act as a potential source of SH (du Vigneaud, Dyer, and Harmon '33; Hammett and Padis '36: Hammett '29).

The first lot purchased contained some 7 per cent of this contaminant. It was discarded as unfit for these experiments. The second lot was prepared by Dr. Theo Lavine of The Lankenau Research Institute expressly for this work. Although no sulfur was detectable in this material the amount analyzed was small (0.2 gram). It is possible the method of analysis was inadequate to determine the very small amount of sulfur which as SH is capable of producing proliferation enhancement. Thus 0.1 per cent or 0.0002 gram would in the M/100,000 culture yield 1.32×10^{-9}S as SH. This amount might give a mild fillip to proliferation.

The way in which leucine and isoleucine seem to associate methionine to themselves in their isolation from protein suggests the existence of a hitherto unsuspected special chemical association of possible importance. Leucine and isoleucine may perhaps be intermediaries for the bringing to expression of methionine function. It may be that the isoleucine used here was sulfur-free and that an affinity thereof for methionine or some cognate compound gave rise to sufficient SH for the mild stimulation of proliferation expression. And then of course it may be that isoleucine is significant *per se* to some phase of proliferation. The solution awaits furthur and different experimentation.

It may seem as if too much attention has been paid to these matters in view of the extreme mildness of the proliferation reaction. Yet pure compounds are essential if true evaluation of their function is to be attained; the presence, nature, and amount of contaminant is important when dealing with concentrations effective at 1×10^{-9}; and persistent associations must have some significance.

Attention has already been drawn to the fact that the addition of CH_2 to the molecule seems to determine an increased regression (Hammett and Schlumberger '37). Thus regression with d-glutamic acid (Hammett '36c) is greater than with l-aspartic (Hammett and Schlumberger '37); it is greater with d-alanine (Hammett '36) than with glycine (Hammett '36a); and it is more affected by thymine (Hammett and Lavine '37) than by cytosine (Hammett, Lavine and Lavine '37).

Here again the phenomenon appears. In every instance save at M/12,500 both by single experiment and by the experiments combined into a single percentage value for each concentration, regression was

increased more by d-isoleucine than by d-valine. And d-isoleucine has one more CH_2 group than d-valine.

Grand totals show the difference. Thus—in 68 experiments with d-isoleucine over the 7 concentration ranges from M/200,000 to M/3400—regression was increased in 40 and decreased in 22; while in the 69 comparable experiments with d-valine regression was increased in 28 and decreased in 28. That is to say, of the deviations from control 40 or 65% were towards increased regression in the d-isoleucine cultures, while but 28 or 50% were in this direction in the case of d-valine (Hammett and Comee '38). The same direction of difference is shown when the data are combined into a single percentage value. Here increased regression due to d-isoleucine amounted to 28.9 per cent while that due to d-valine was only 0.3 per cent.

The persistence of this enhanced reaction accompanying the introduction of CH_2 into the molecule through 4 pairs of compounds is evidence enough of its validity and the validity of allocation to CH_2 of a specific regression increasing influence.

Whether or not this finding will be found related to that of Leighty and Corley ('37) that adding methyl groups to amino acids interferes with their deamination, and that of Polonovski and Boulanger ('38) that increasing the length of the amino acid chain decreases its effect on ammoniophaneresis has yet to be ascertained.

Regardless of these or other possible relationships the significant thing is that it is possible to allocate a specific function to a specific chemical group. The nature of this will be gone into in more detail in a later paper.

Again attention is drawn to the fact that no other amino acid than d-isoleucine has the combined effect on the growth and metabolism of obelia which is described in preceding paragraphs. This again sustains the postulate that each of these naturally occurring tissue components of general distribution has its own specific function as a growth determinant.

SUMMARY AND CONCLUSION

Eighty experiments with close to 38,000 animals (*Obelia geniculata*) half of which were exposed to d-isoleucine in concentrations ranging from M/400,000 to M/3400 in two seasons with two different sets of workers show that the compound,—

1. Has a definite inhibitory effect on the developmental activity of initiation which is perhaps primary on some process specific to anlagen productivity.

2. May have a slight forwarding effect on the developmental activity of proliferation which possibly is due to contamination with a sulfur carrying amino acid.

3. Has no effect on differentiation or organization as developmental activities.

4. Tends to forward growth of hydranths in general; but not of gonophores.

5. Tends to increase regression in the non-toxic concentrations. May have a specific dynamic action.

6. May act to retard catabolism slightly.

7. Is toxic at M/50,000.

8. Is more stimulative of regression than is d-valine.

The conclusion is that insofar as growth is concerned d-isoleucine is a participant in some preparatory process essential to new structure production, and that insofar as metabolic activity is concerned the added CH_2 is a specific factor in regression of the complete hydranths.

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NUTRITION VS. HEREDITY IN DETERMINING STATURE
(A Review)

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INTRODUCTION

The adult individual is the result of an interplay between his genetic endowment and his environment. Heredity determines very definitely the potentialities of an individual in various directions both physical and mental, but other factors more within the scope of man's control may determine whether or not these potentialities are fully achieved. Nutrition is very definitely one of the more important of these environmental factors which affects among other things the stature or ultimate size of the individual. Of course, the classical question "Is the bigger baby the better baby?" is, as yet, unanswered. Happiness, usefulness and even success in life are by no means dependent on the height and weight of a person. Still a fine physical development is generally considered a valuable asset in this business of living.

There can be no doubt that nature has set an upper limit to stature. The fact remains, however, that we have no exact way of knowing what nature's limitations are in any specific case. Because a person grows to a certain size even though it be that attained by his immediate ancestry, what proof is there that he has reached the limits nature has set for him? How much taller and heavier both the undersized child and his father would be if they were better fed. Much is blamed to heredity which can be explained in other ways. It is not always "fatness" as such that runs in families; this trait might better be attributed to the fact that families usually eat at the same table.

The limitations in the methods of investigating some studies of stature may have tended to obscure or minimize the importance of food. Size of parents and of grandparents will usually be accepted as clear cut quantitative data but it is wholly impossible to obtain equally quantitative data as to the food consumed in a lifetime.

AMERICAN STUDIES

Physicians in this country seem generally agreed that the present generation of children are taller and heavier than their parents owing to better infant and childhood feeding. This viewpoint is in keeping with the results of several investigations made both in America and in other countries. Bowles (1936) compiled the anthropometric measurements of fathers and sons who attended Harvard University; and mothers and daughters at Vassar, Wellesley, Smith and Mount Holyoke. At Harvard data on 1942 couples (fathers and sons) were obtained. Racially the subjects were remarkably constant only 5.79 percent being other than "old American" stock. Sons are absolutely larger than their fathers, showing a marked average increase of two inches in height and seven pounds in weight. Even though they exceed parents in absolute weight, men today are getting taller and slenderer. The mean stature of the present day "old American" student population of Massachusetts and vicinity is 178.03 cm. rating thus among the tallest people observed anywhere and 4 cm. above the population as a whole.

The daughters increased 2.93 cm. in height and four pounds in weight over their mothers at the girls' colleges studied. Bowles offers as one of the probable contributing factors for this difference better food in more abundance and in greater variety.

A similar study was conducted by Jackson (1931) on female students at the University of Minnesota. This was an 18 year period investigation on 16,786 students. Annual numbers increased from 400 in 1912 to 1393 in 1929 and although the mean age decreased from 20.36 to 20.48 years during this time, the mean height increased 0.1 inch and the mean weight 2.35 pounds. This evidence does not support the theory of an artificial decrease in the body weight of college women to conform with female fashions of recent years.

At the same university MacKinnon and Jackson (1931) compared measurements of 1205 male students of the period 1898 to 1901 with those of male entrants in 1929. The results here also show an improvement in stature: 0.8 inches in height; 3.9 pounds in weight and 168 cc. in vital capacity. These figures are still more impressive because of the corresponding decrease of 1669 years in age for the total group. Gray (1927) reports that American born boys of American born parents are taller by two inches today than similar boys of fifty years

ago. This statement is the result of findings at Massachusetts Institute of Technology, Boston Public Latin School, and Private Latin School on 303 Boston boys compared with data on Boston boys attending equivalent class schools 50 years ago.

OTHER STUDIES

The improvement in size is not confined to American students alone. There seems to be a measurable increase in the stature of any people who improve their national diet or who move to another country and become adjusted to a better diet.

The second and succeeding generations of immigrants to the United States from Central Europe or Japan are taller, have larger frames and are more robust than their parents. The higher standard of living with its wider variety and choice of food finds immediate response in the physical development of these people. The children of successful Russian Jews who have become Americanized to a considerable degree are four to five inches taller than their parents.

Steggerda (1932) found that in span and head size American Dutch are larger than European Dutch. This study was made on 130 inter-related Dutch men and women, half of whom lived in the Netherlands and half of whom lived in a Dutch community in Michigan.

Appleton (1927) (1928) found that Cantonese adults in Hawaii are taller then Cantonese adults in China and the growth process of Chinese in a favorable environment resembles the growth process of Europeans more than of Chinese in an unfavorable environment but body proportions remain distinctive to the race. From this one would infer that good nutrition would change a whole race of peoples markedly.

The post-war period provided interesting material for studies of this nature. In Germany Kuhn and Breda (1926) found that the stature of German students in general improved gradually under the influence of ameliorating conditions of 1923-1925.

The same was true in Russia where 65,790 children were studied by Newsoroff (1927). The mean growth of late post-war children (1925) was greater than that of the pre-war child. Poor living conditions and lack of nourishment are the reasons given for the difference although improvement is not yet stabilized.

It is well-known that diets of certain Oriental peoples are not suitable for proper nutrition. The people of the Shantung Peninsula area in

the north of China are fine specimens of physical development. Here the whole grain of wheat is eaten instead of the polished rice used by the smaller southern Chinese. The degerminated and decorticated polished rice might make a sufficient difference in the mineral content of the diet to account for the relative decrease in stature.

At the beginning of the century there was a great influx of "picture brides" from Japan into California and other Pacific Coast cities resulting in a large number of American born children in this country of Japanese parentage. Both boys and girls are larger at all ages than are Japanese children born and reared in Japan in spite of the climate of Japan being more stimulating than the climate of California. This difference is represented in Table 1, which is a summary of charts compiled by the Children's Bureau of U. S. Department of Labor; the Japanese Educational Association of America; and the Educational Department of the city of Tokio and used by Kanzaki (1921) in his argument for Japanese immigration.

TABLE 1
COMPARATIVE HEIGHTS AND WEIGHTS OF JAPANESE CHILDREN

	Born and Reared in America			Born and Reared in Japan	
Sex	Age	Height	Weight	Feet	Pounds
	Years	Feet	Pounds	Height	Weight
Boys	7	3.54	42.3	3.52	38.6
Girls	7	3.54	40.4	3.48	37.3
Boys	12	4.38	65.4	4.25	60.
Girls	12	4.35	65.4	4.24	59.5
Boys	16	5.2	103.5	5.1	98.5
Girls	16	4.95	105.6	4.8	95.2

Stature standards of Philippine students are lower than those of students in other countries. This is attributed not so much to the lack of food as it is to the improper balance of diet.

Controlled growth studies of children in schools in England, Scotland, Norway, the United States and many other civilized countries show the same results as do stature studies on adults. In general where there is improved nutrition, there is an increased growth rate.

THE ROLE OF DIETS

Anthropometric measurements alone would be invalid in a study of nutrition and stature relationship if specific diets were not considered too. Good physical development seems to prevail in those countries where dairy products are most available to the inhabitants. The pastoral nomads, for example, the Bedouins and Jews of Arabia, are illustrative of a people of fine physical attainment, subsisting under adverse conditions of climate and aridity. Their size is above the average of men in general. The diet of these pastoral peoples consists in a great measure of milk with moderate amounts of meat, cereal, dates, pomegranates, and melons. The use of very liberal amounts of milk is the rule among the people of Northern Africa, Mesopotamia, and the Balkan States. Wherever dairy animals are abundant in proportion to population, a good physical development is seen without exception.

True, the American born Japanese children, cited earlier, though taller than Japanese children, are not as tall as native Americans but neither is their diet a completely typical American one. In the brief course of one generation, it is difficult to break dietary customs that date back centuries. The usual Japanese diet is composed chiefly of rice, some fish but no meat, green vegetables in summer, and much sweets. Any fat used is vegetable fat and dairy products are rare. This type of diet would seem clearly deficient in growth promoting proteins and probably in the fat soluble vitamins and calcium.

In the United States there must have been a noticeable change in the average diet to promote the increased height and weight of present day college students over their predecessors. Since 1900 statistics show the per capita consumption of fresh and canned fruits and vegetables and dairy products has been increased. Oranges alone have more than doubled in fourteen years. It is the concensus of opinion that many people are still obtaining less than optimal amounts of the water soluble vitamins, yet sources of these are so abundant that, in general, a rational dietary instinct would lead inevitably to their selection.

The League of Nations Committee (1937) is also of the opinion that stature improvement is unquestionably related to food consumption habits. In England a study was made of the diets of the various income groups. The conclusion reached was that the average diet of the 10 percent population with lowest incomes was inadequate for perfect

health in all the main essential constituents; the next higher 10 percent group diet was found adequate only in total proteins and table fat; the next 20 percent adequate in energy value, protein and fat but below standard in calcium. There was an ample margin of safety found in the next 20 percent group's diet in everything except calcium; and only in the 10 percent top income class were the standard food requirements exceeded in all the essentials. Is it any wonder that stature studies show private school students to be taller and heavier than public school students of lower income groups? It is also not surprising that with the newer knowledge of nutrition, the present generation in America, England and other countries where such studies have been conducted is taller and heavier than preceding generations.

CONCLUSIONS

The results of these investigations seem to prove the theory that as hereditary patterns can be modified by conditions of life (nutrition), it ought to be possible to improve the physical status of man.

Dr. T. W. Todd (1935) interpreted most admirably the results of anthropometric measurements in relation to good nutrition: "The adult form of mankind is the outcome of growth enhanced, dwarfed, warped or mutilated by the adventures of life. I am not decrying heredity. But I do insist that new knowledge gained for anthropology through the study of growth brings hope and confidence where formerly was only the grim figure of destiny."

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THE GROWTH ACTIVATING EFFECT OF EXTRACTS OF ADULT TISSUE ON FIBROBLAST COLONIES IN VITRO

EXPERIMENTS WITH CHICKEN HEART EXTRACTS

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The usual technique of tissue culture is built on the use of extracts of embryonic tissue as a basic constituent of the culture medium. As distinguished from plasma—another basic constituent of culture medium—embryonic extract has the property of stimulating cell-growth to a marked extent.

It is now generally accepted that "embryo extract alone, of all tissue extracts, is capable of exerting these great growth promoting properties" (E. Willmer (1)). Only extracts of embryonic tissues are thought to contain growth promoting substances in large quantities (G. Levi (2), J. Verne (3)).

Extracts of tissues and organs from adult animals have also been used occasionally as a constituent of tissue culture medium. In this case, however, not extracts of stable tissue but extracts of mitotically active—neoplastic—tissues or of tissues with active cell formation (e.g. bone marrow), were mainly employed. (A Maximow (4), M. Mollendorff (5), and others). The growth-promoting activity of these extracts is regarded as smaller than of extracts of embryonic tissues (E. Willmer, G. Levi).

One might conclude from the above that in general growing tissues and tissues with active cell formation in contrast to nonproliferating tissue are capable of elaborating cell-growth promoting principles in appreciable quantity. In order to test the validity of such a conclusion, experiments were carried out comparing the growth promoting properties of extracts of mitotically active tissue with those of non-growing tissue.

We considered these experiments particularly appropriate, as the widespread conception that growth promotion is chiefly a property of growing tissue is not only lacking experimental basis but is also

definitely at variance with experiments already carried out in this field.

It must be stressed that A. Carrel, who introduced the use of embryonic extract into tissue culture technique, never claimed an exclusive stimulating effect for this extract on mitotic activity of cells in vitro. He emphasized only the fact that the presence of embryonic extract was necessary for the *permanent* cultivation of cells. Actually Carrel showed in experiments carried out in 1913 that extracts of different tissues of adult animals stimulated growth of connective tissue in vitro.

A. Carrel (6). tested extracts of spleen, liver, connective tissue, kidney, heart, blood corpuscules, *thyroid gland, and muscle and found that the degree of activation varied considerably with the kind of tissue used. Extracts from adult spleen were very active; kidney and heart extracts were less active; and those of connective tissue and blood corpuscules were still weaker. This stimulating effect of extracts of adult tissue on growth of tissue cultures was shortly thereafter confirmed by A. Walton (7) and reaffirmed by A. Carrel and A. Ebeling (8).

These much overlooked, though evidently important, experiments were in the main performed when tissue culture technique was still in its initial stage and did not permit of exact quantitative analysis of the problem. The experiments reported in this paper rest on early findings and tend to render them more conclusive.

MATERIALS AND PROCEDURE

Extract of cardiac muscle of adult chicken was used to study the growth promoting effect of stable tissue. This was compared in activity with extract of mitotically active tissue—7 day chick embryo extract.

There is only one reference in the literature to the growth activating effect of cardiac muscle: A. Carrel (6) reports that heart extracts are much less active than extracts of embryonic tissues, adult spleen, of Rous Sarcoma, and more like kidney extract.

Preparation of Heart Extract—The heart is removed from one year old chicken under sterile conditions; the fatty tissue is cleaned away and the heart then finely sliced with a Latapie apparatus. Tyrode solution is added to the tissue pulp in the proportion 1:8 and the

* Leucocytes to which Carrel ascribed pronounced ability to elaborate growth promoting substances occupy an exceptional position.

TABLE I

SIZE VALUE OF 6 DAY CULTURES GROWN IN PLASMA TYRODE + CHICKEN HEART EXTRACT MEDIUM

Culture No.	Size of culture in mm²
4b	55
5a	40
5b	33
6a	41
6b	50
100a	53
100b	60
101a	46
101b	53
101c	68
101d	63
102a	50
102b	55
103a	54
103b	50
103c	75
104a	56
104b	65
104c	65
106a	68
106b	67
107	58
108a	85
108b	56
109a	66
109b	56
110a	51
110b	68
225a	28
225b	28
223a	30
223b	45
224a	45
224b	38
229a	38
229b	44
230a	66
230b	44
231a	35
231b	36
232a	85
232b	75
233a	39
233b	44
234a	53
234b	41
235a	50
235b	50
238a	34
238b	40
	Mean: 53.9 mm²

TABLE II

SIZE VALUES OF 6 DAY CULTURES GROWN IN PROTECTIVE (PLASMA-TYRODE) MEDIUM

Culture No.	Size of culture in mm²
284a	21
284b	14
285a	19
285b	16
286a	16
286b	14
291	14
307a	14
307b	14
308a	7
308b	15
310a	14
310b	11
310c	14
311a	7
311b	10
311c	11
312a	7
312b	15
312c	19
313a	10
313b	11
326a	14
326b	20
327a	10
327b	19
328a	11
328b	11
329a	4
329b	7
329c	13
336	15
337a	13
337b	14
337c	20
338a	11
338b	12
339a	8
339b	14
340a	8
340b	10
340c	12
341a	15
341b	16
341c	19
342a	4
342b	6
342c	9
343	11
347	12
	Mean: 12.4 mm²

mixture is well shaken. After standing for 1 hour in the refrigerator the mixture is centrifuged for ten minutes and the supernatant fluid removed. The extract is kept in the refrigerator and remains in good condition for weeks.

Tissue Culture Technique—The stimulating effects of tissue extracts were tested on fibroblast colonies in Carrel flasks. Flask cultures were prepared as follows: A hanging drop culture, usually a 3rd passage, was divided into two equal parts: One culture half was used as a control in a flask containing chicken plasma diluted 1:2 with Tyrode. To the other half in the same plasma-Tyrode medium, 12% heart extract was added as fluid phase. In some experiments 12% embryonic extract was added as fluid phase to the controls.

The fluid phase was not changed during the entire course of the experiment. Growth was observed for a period of from 6-8 days. Growth curves of the control and experimental cultures were constructed by planimetric measurements of the area of the culture surfaces after the method of Ebeling.

EXPERIMENTS AND RESULTS

1. *Stimulating Effect of Heart Extract*—The results of our experiments on the growth activating property of medium containing cardiac muscle extract are summarized in Tables I and II. Table I gives the size of mm^2 of fibroblast colonies after 6 days' growth in vitro in media containing cardiac muscle extract; Table II gives the corresponding size of fibroblast colonies after 6 days' growth in a protective medium (plasma-Tyrode).

The addition of cardiac muscle extract to culture media causes a very marked increase in growth rate of fibroblast colonies.

The mean value for the area as calculated from fifty 6 day old fibroblast cultures in protective medium is 12.4 mm^2; the corresponding value of 6 day old cultures in media containing heart extract is—53.9 mm^2.

The curves of Fig. 1 demonstrate clearly the stimulating effect of heart extract in two experiments. They represent the growth curves of two fibroblast cultures of which sister halves were planted respectively in protective medium and in medium containing heart extract. Cultures grown in medium containing heart extract, and in protective medium are shown in figs. 2A and 2B.

The structure of fibroblast cultures growing in medium containing heart extract is very uniform and regular. The growth is always luxuriant and the cell colony of uniform thickness, and rarely thin at the periphery (Fig. 3). The cells are slightly granulated.

2. *Comparison of Stimulating Action of Heart Extract and Embryonic Extract*—Comparative experiments showed that under the conditions chosen *the cell-growth stimulating action of heart extract is far greater than that of embronic extract.*

Table IV indicates the surface area in mm² of cultures grown for 6 days in medium containing embryonic extract. Whereas the mean area

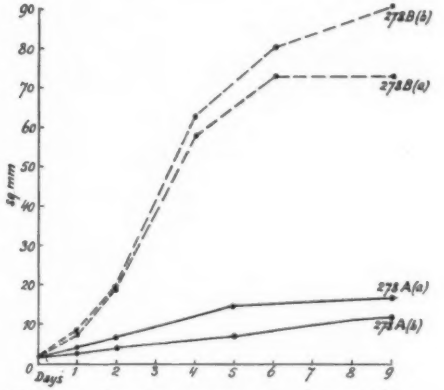


FIG. 1 Growth curves of sister halves of fibroblast cultures in heart extract medium and in protective (plasma Tyrode) medium.
278 A(a) and 278 A(b) Cultures in protective medium
278 B(a) and 278 B(b) Cultures in medium containing heart extract

TABLE III
(TO FIGURE I)
SIZE OF CULTURE IN MM²

No. of Day	Culture No. 278 A (a)	Culture No. 278 A (b)	Culture No. 278 B (a)	Culture No. 278 B (b)
1st	0.5	1.5	0.5	1.0
2nd	2.0	3.5	7.0	8.0
3rd	3.5	6.0	18.75	18.75
5th	5.0	12.0	57.5	62.5
7th	8.5	14.0	72.5	80.0
10th	11.0	16.0	72.5	90.0

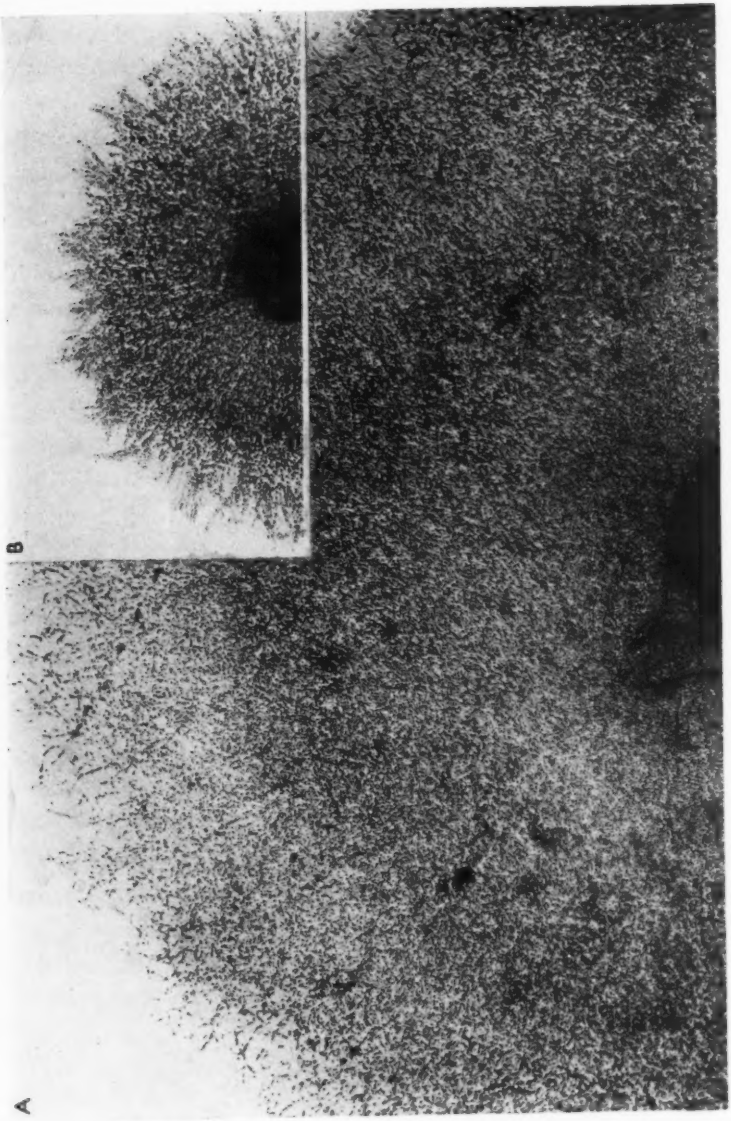


FIG. 2 (A) Nr. 285 B 8 day old fibroblast colony in heart extract medium. X 20. living
(B) Nr. 285 8 day old fibroblast colony in protective (plasma + Tyrode) medium. X 20. living

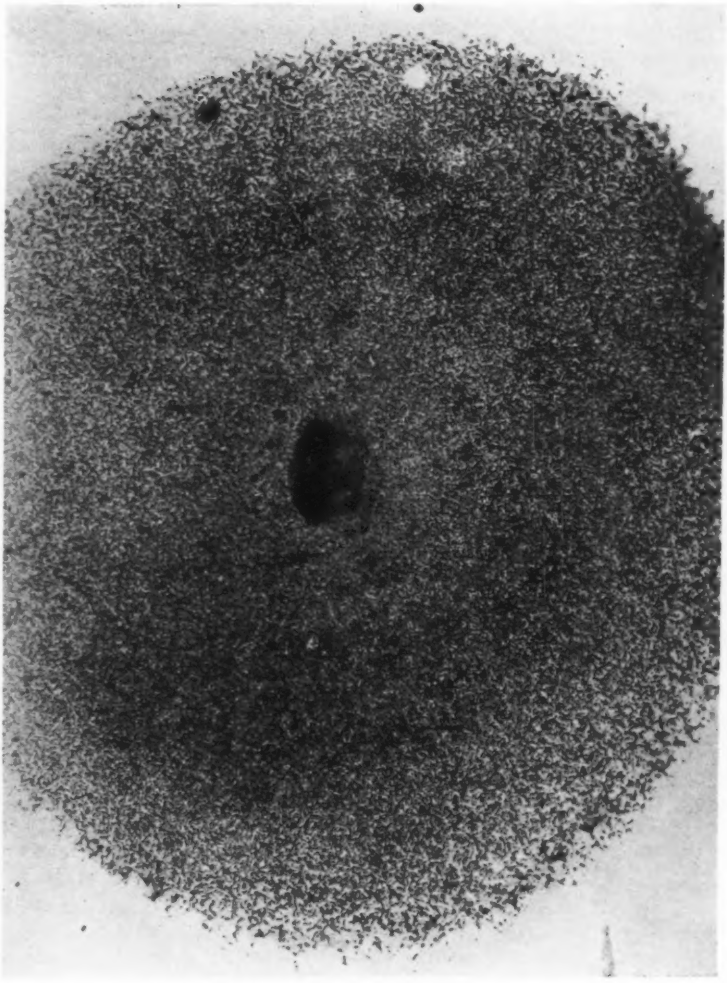


FIG. 3 Nr. 477a 7 day Fibroblast colony in heart extract medium X 12 living

of fifty cultures stimulated by heart extract is 53.9mm^2, that of cultures in embryo extract is 19.7 mm^2; within this time a few rare colonies in embryo extract attained a size of 40-44 mm^2, but in heart extract some cultures had double this area. The growth curves of 3 culture pairs in heart extract and embryonic extract are presented in Fig. 4—the

behaviour of the cultures, one sister half of which is cultivated in extract of heart muscle, the second in embryonic extract—in Table VI. The various growth rates of cultures in protective, embryonic, and heart extract media are clearly shown in Fig. 5.

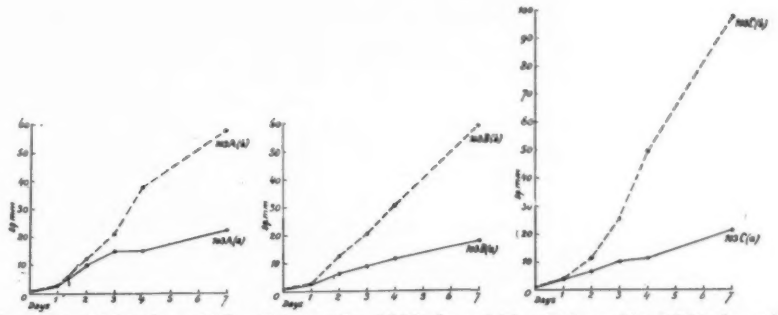
TABLE IV
SIZE VALUE OF 6 DAY CULTURES GROWN IN PLASMA-TYRODE MEDIUM+ CHICKEN EMBRYO EXTRACT

Culture No.	Size of culture in mm^2	Culture No.	Size of culture in mm^2
4a	19	212b	8
4b	12	219	19
6a	13	223	15
6b	13	224	3
100	30	225	4
102a	13	229	10
102b	13	230c	31
101	21	230d	34
103a	20	231	19
103b	15	232	19
104a	26	233c	33
104b	26	233d	44
107a	28	234	16
107b	23	243	6
109a	33	244a	29
109b	35	244b	26
110	21	245	12
200	5	246	9
201	19	291a	25
204a	3	291b	31
204b	24	291c	25
207	8	284	40
208	24	285	34
211	10	286c	11
212a	13	286d	18

Mean: 19.7 mm^2

TABLE V
(TO FIGURE 4)
SIZE OF CULTURE IN MM^2

No. of Day	Culture No.103A(a)	Culture No.103A(b)	Culture No.103B(a)	Culture No.103B(b)	Culture No.103C(a)	Culture No.103C(b)
1st	0.5	0.5	0.5	0.5	0.5	0.5
2nd	2.5	2.5	2.5	2.5	3.75	3.75
3rd	10.0	12.5	6.25	12.0	6.25	11.0
4th	15.0	21.25	8.75	21.2	10.0	25.0
5th	15.0	37.5	11.25	31.2	11.0	48.75
8th	22.0	57.5	17.5	58.75	21.25	97.5



Culture No. 103A (a and b) Culture No. 103B (a and b) Culture No. 103C (a and b)

FIG. 4 Growth curves of sister halves of fibroblast cultures in heart extract and in embryonic extract media.

-------- Culture in heart extract medium

——— Culture in embryonic extract medium

TABLE VI

ACTION OF ADULT HEART EXTRACT AND OF EMBRYO EXTRACT ON THE RATE OF GROWTH OF CHICKEN FIBROBLAST

(SISTER PAIRS)

HE=HEART EXTRACT

EE=EMBRYO EXTRACT

Culture No.	Medium	Size in mm² after days			
		1st	3rd	6th	8th
353a	EE	0.8	6.7	18.7	21.7
354a	HE	0.7	6.8	32.0	46.4
353b	EE	0.6	5.7	12.1	15.4
354b	HE	1.1	21.7	68.0	85.0
355a	EE	7.5	4.9	4.0	19.9
356a	HE	0.8	11.7	52.3	58.7
355b	EE	1.6	16.1	41.0	54.8
356b	HE	1.2	19.7	79.4	101.8
357a	EE	0.8	7.4	17.6	22.4
358a	HE	1.0	17.4	69.6	79.6
357b	EE	0.6	9.0	21.0	26.4
358b	HE	0.9	15.8	47.4	55.8
359a	EE	1.9	8.1	54.0	63.6
360a	HE	1.4	53.6	64.2	82 1
359b	EE	0.9	10.7	26.4	26.9
360b	HE	1.0	22.8	64.1	68.9
361a	EE	2.9	7.4	19.2	25.1
362a	HE	0.6	14.9	48.4	63.1
361b	EE	1.1	10.9	24.8	35.7
362b	HE	1.1	23.1	62.3	77.2
363a	EE	0.6	8.7	17.8	21.1
364a	HE	0.8	15.6	40.3	52.5
363b	EE	1.3	8.9	19.5	22.6
364b	HE	1.3	24.4	85.8	96.4

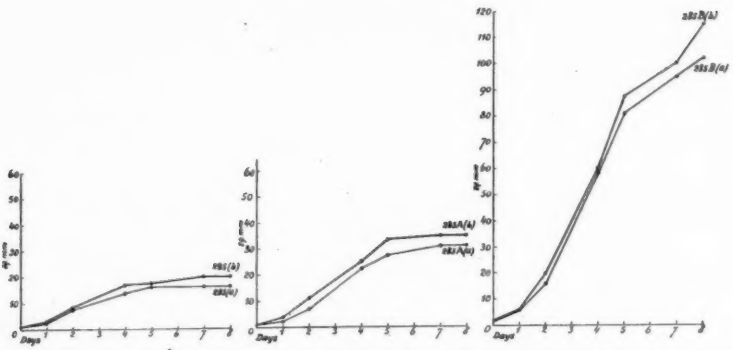


Fig. 5 Growth curves of fibroblast cultures in plasma-Tyrode, embryonic extract and heart extract media.

No. 285 (a) and 285 (b)—Culture in protective medium
No. 285A (a) and 285A (b)—Culture in embryonic extract medium
No. 285B (a) and 285B (b)—Culture in heart extract medium

TABLE VII
(TO FIGURE 5)

No. of Day	SIZE OF CULTURE IN MM^2					
	Culture No.285(a)	Culture No.285(b)	Culture No.285A(a)	Culture No.285A(b)	Culture No.285B(a)	Culture No.285B(b)
1st	1.25	1.25	1.25	1.25	2.0	2.0
2nd	2.5	3.0	2.5	3.75	6.25	6.25
3rd	7.5	8.0	7.0	11.0	15.6	20.0
4th	13.75	17.0	22.5	25.0	59.0	60.0
5th	16.3	17.5	27.5	33.75	81.0	87.5
6th	16.9	20.0	31.3	35.0	95.0	100.0
7th	16.3	20.0	31.3	35.0	102.0	115.0

CONCLUSIONS

These experiments show that extract of stable, mitotically inactive, tissue like extract of growing tissue is capable of exerting marked growth promoting properties.

Aqueous extract of cardiac muscle of adult chicken strongly stimulates the growth of chicken fibroblast colonies in vitro.

Under the conditions of the experiments, the stimulating effect of heart extract was found to be greater than that of embryonic extract.

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BOOK REVIEW

METHODS OF TISSUE CULTURE. Raymond C. Parker, with a foreword by Alexis Carrel. Paul B. Hoeber, Inc. New York. 1938. XXXII + 292 pp.

This is more than a mere handbook. It is an excellently written and beautifully printed *mise au point* of the entire field of animal tissue culture so much of whose development has taken place in Dr. Carrel's laboratory. Dr. Parker has described the tissue culture laboratory, the preparation of materials, experimental manipulations, and methods of interpreting results in detail, with very helpful illustrations. He has then gone on to discuss examples of the work done in various branches of the field. If the reader will peruse the book's pages carefully and make full use of what is set before him, he should not only be able to make use of this important technique but should be in a position to distinguish the more important lines of approach and avoid repetition of work already done. Moreover, the book is far from dry reading. It is interestingly written and full of little side lights that place it above the level of the usual manual. And, so far as this reviewer can judge, it avoids the many errors, due to careless writing, that so marred Albert Fischer's earlier "Gewebezuchtung". The selected bibliography is excellent and the photographs really worthy of the highest praise. This reviewer found it impossible, from the meagre details given, to visualize the functioning of the Lindbergh "Gas-lift flask" (pp. 122-123) and, since the details seem not to have been published in the current literature, he will presumably have to remain in the dark on this score. Diagrams or photographs of the best types of apparatus for taking time-lapse micro-cinemas of tissue cultures might well have been included. But these are minor lacunae.

There is one criticism which this reviewer would like to make, not of this book in particular, but of the entire animal tissue culture field in general. The bibliography appended to Dr. Parker's book, including titles of 753 books and papers, is probably a fair cross section of the literature of the field. It is divided into 13 sections, one of which is "Physicochemical constitution of the medium". This includes 60 titles or about 8% of the total. And only 4 of these sixty—half of one per cent of the total—bear titles indicating that they deal with inorganic constituents of the nutrient, and one of these four does so only incidentally. A careful review of the other sections discloses seven additional scattered papers that may possibly refer to this subject. But the surprising fact remains that only 3 out of the 753 references selected by Dr. Parker appear to deal in major part with the effects of inorganic nutrients on animal tissue cultures. It is hard to believe that only one half of one per cent of the problems that arise in animal

tissue development, or that can be approached by the method of studying excised tissues, involve or are influenced by the salts contained in the nutrient. Most of the field seems to have been well worked but here is one corner in which, it seems, a few keen prospectors might still find pay dirt!

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